J.Cell.Mol.Med. Vol 5, No 2, 2001 pp. 153-162

The hyperlipemic hamster - a model for testing the anti-atherogenic effect of amlodipine

Anca Sima, Camelia Stancu, Elena Constantinescu, Laura Ologeanu, Maya Simionescu *

Institute of Cellular Biology and Pathology "Nicolae Simionescu", Bucharest, Romania

Received: June 12, 2001; Accepted: June 25, 2001

Abstract

Male Golden Syrian hamsters were subjected to a hyperlipemic diet. At intervals ranging from 2 to 14 weeks, the animals were examined for changes in serum constituents and structural modifications of lesion-prone areas: the cardiac valves, coronary arteries and aortic arch. Serum was characterized by a gradual increase in cholesterol, triglycerides and a decrease in total peroxyl-radical trapping potential. The sequence of modifications of the endothelial cells, smooth muscle cells, and migrating plasma monocytes as well as of the extracellular matrix were established. Amlodipine treatment of hyperlipemic hamster was assessed. Amlodipine exhibited an athero-protective effect, acting as antioxidant, reducing the LDL uptake by the vessel wall and consequently, limiting the size and extent of lesioned areas. The hyperlipemic hamster is a reliable model to unravel the cellular alterations leading to atheroma formation, and for testing the effect of drugs in this process.

Keywords: amlodipine - hyperlipemia - atherosclerosis - hypercholesterolemic hamster - fluorescent LDL - lipoproteins - electron microscopy

Introduction

In humans, atherosclerosis is a focal disease that has been shown to evolve in a distinct pattern, leading to atheroma formation and vessel obstruction [1, 2]. Given the complexity of lesion development, the sequence of events that take place is difficult to be assessed in man. The challenge exists to develop suitable animal models that will closely mimic the human disease. Although there is no perfect animal model, they are useful to study sequentially the pathologic alterations, from the inception to the final stage of the atherosclerotic plaque. Irrespective of the species, the induction of vascular lesions is dependent upon hyper-cholesterolemia. Plasma cholesterol elevation can either be induced by dietary supplementation, hepatic overproduction of lipoproteins or genetic mutation of receptors and/or receptor ligands responsible for lipoprotein clearance.

The Golden Syrian hamster was successfully used to investigate vascular changes that take place

*Correspondence to: Dr. Maya SIMIONESCU
Institute of Cellular Biology and Pathology "Nicolae Simionescu",
8, B. P. Hasdeu Street, Bucharest 79691, Romania.
Tel.: (+401) 411.5240; Fax: (+401) 411.1143.
E-mail: simionescum@simionescu.instcellbiopath.ro

in atherogenesis [3]. As compared to other animal models, the hamster has several advantages: i) as in humans, the main plasma cholesterol carrier is LDL and the lipoprotein metabolism displays similarities to that in man [4], ii) the hamster LDL receptor gene has been isolated and characterized [5] and shows strong similarities to the human gene, iii) atherosclerotic plaques develop with predilection in aortic arch, the aortic aspect of the sigmoid valves, coronary arteries (lesion-prone areas), allowing reliable assessment of atherosclerotic process.

Amlodipine (Aml), a calcium channel blocker (CCB), is a highly lipophilic molecule, used successfully in hypertension treatment; there are also reports that Aml is an important atheroprotective agent, acting as a powerful antioxidant [6, 7], lacking significant pro-oxidant properties [8].

Low density lipoproteins (LDL) oxidation is believed to play an important role in atheroma formation [9, 10, 11, 12, 13, 14]. In hyperlipemic hamsters the inception of the disease is linked to the presence of circulating modified LDL and their increased uptake by the arterial wall [15].

The aim of our study was to characterize the ultrastructural modifications and functional alterations (by the uptake of LDL) in pro-atherogenic areas, and the possible protective effect of Aml treatment in hyperlipemic hamsters. We provide evidence that Aml effectively reduces uptake of LDL by the arterial wall and diminishes lesion formation.

Materials and methods

Reagents

Enzymatic kits for total cholesterol and triglycerides determination and 2,2'-diazobis (2-amidinopropane) dihydrochloride (AAPH) were purchased from Sigma-Aldrich Chemie GmbH, Germany; 2,7-dichloro-fluorescein-diacetate (DCFH-DA) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid (Trolox) used for serum total peroxyl radical trapping potential (TRAP) procedure were obtained from Fluka Chemie AG, Germany; 1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate (DiI) were purchased from the Molecular Probes, Inc. Eugene, OR, USA.

Amlodipine (3-ethyl-5-methyl-2-(2-aminoethoxy-methyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine-dicarboxylate benzenesulphonate) was extracted from Norvasc tablets (Pfizer, USA). All other reagents were of analytical grade.

Animals

80 adult male Golden Syrian hamsters (100- 140g body weight), kept in standard conditions with free access to water, were divided in three groups: (i) hamsters fed with standard diet supplemented with 3% cholesterol and 15% butter (HH); (ii) hamsters fed with the same diet as for HH treated daily by gavage (14 weeks), with 0.1 mg/kg body amlodipine (HA), the chosen dose corresponding to the 10 mg Aml tablet administered to patients; (iii) hamsters fed with standard diet used as control (HN).

Biochemical assays

Every two weeks, blood was collected from the retro-orbital plexus of fasted animals; cholesterol, triglycerides, and total peroxyl radical trapping potential [16] were determined.

Fluorescent labeling of LDL

Human LDL was separated by density gradient ultracentrifugation from plasma isolated from healthy donors (Haematology Center, Bucharest). LDL was incubated with DiI, a fluorescent (red) compound with excitation at λ_{ex} = 548 nm and emission at λ_{em} = 574 nm, in the presence of delipidated sera, for 18 hours at 37°C [17]. DiI was used because it adds to the lipoprotein similarly with phospholipids and does not affect the binding capacity of the particle to the receptor. After incubation, LDL-DiI was ultracentrifuged in gradient density for 3h at 280,000 xg. LDL-DiI was collected and dialysed overnight in PBS pH 7.4 at 4°C, in the dark.

Tissue preparation for light and electron microscopy

Hamsters were anesthetized and, after laparotomy and catheterization of the abdominal aorta, the vasculature was washed out of blood by perfusing under pressure (100 mm Hg) phosphate-buffered saline (PBS); vena cava was used as outlet. Further, a mixture of aldehydes (2.5% paraformaldehyde, 1.5% glutaraldehyde, 2.5 mM $CaCl^2$ in 0.1 M HCl-Na cacodylate buffer pH 7.4) was perfused and the tissues fixed in situ for 10 min. Then, the aortic arch and heart were collected, and the right atrium, left ventricle, coronary arteries and aortic valves dissected and separately processed. All specimens were subsequently

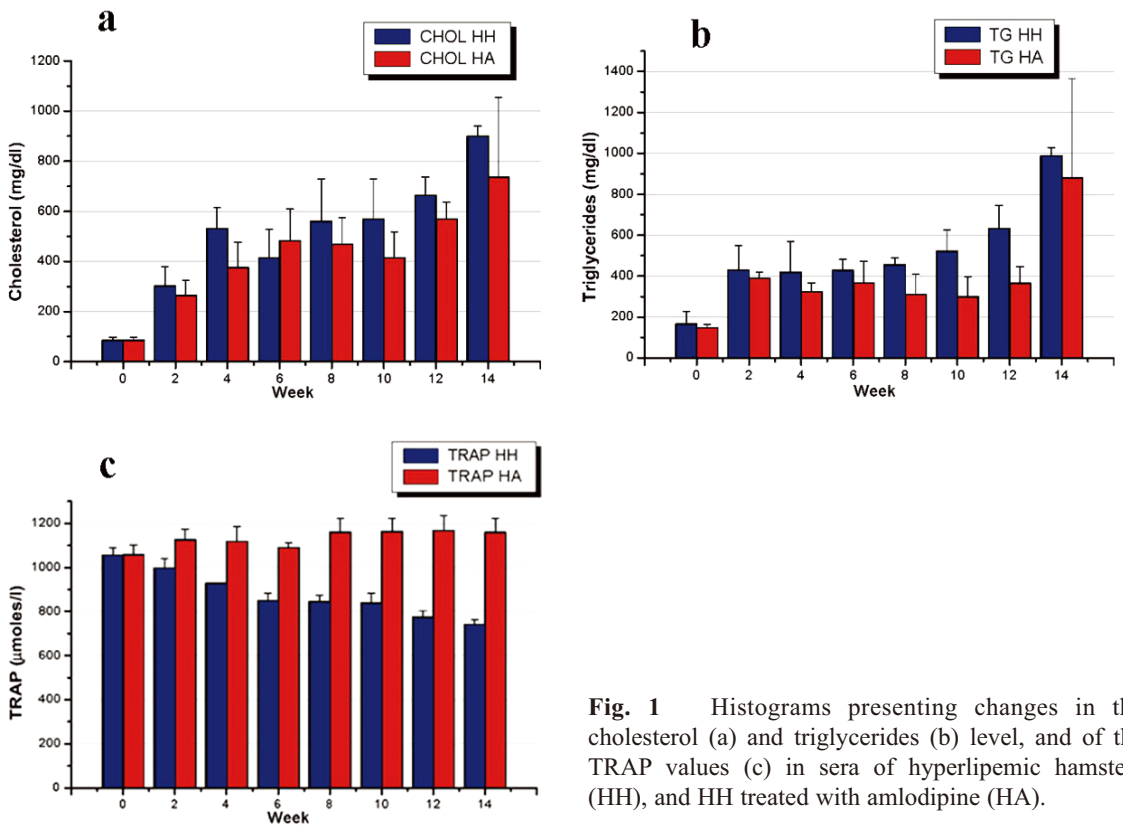

Fig. 1 Histograms presenting changes in the cholesterol (a) and triglycerides (b) level, and of the TRAP values (c) in sera of hyperlipemic hamsters (HH), and HH treated with amlodipine (HA).

treated with 1% OsO^4 in the same buffer, uranyl acetate staining in block, dehydration and embedding in Epon 812.

In some experiments, before tissue fixation, cationized ferritin (CF, 1 mg/ml in PBS) was perfused for 2 min through the vasculature. Afterwards, the standard protocol for EM, as described above, was used. Thick and thin sections, cut with OmU3 Reichert ultramicrotome (Austria), were examined with Philips 400 HM and 201C electron microscopes.

Tissue preparation for fluorescence microscopy

At different time intervals after the beginning of the experiment, LDL-DiI (1 mg protein) was intravenously injected and maintained in the circulation for 24h. Hamsters were anesthetized, and after laparotomy, the abdominal aorta was catheterized and the vasculature was washed out of blood as above. Tissues were fixed by perfusing 2% paraformaldehyde, and after 10 minutes the aortic arch, sigmoid valves and thoracic aorta were collected. After washing with PBS, the specimens were kept in PBS containing 5% sucrose, over night at 4°C. The samples were successively immersed in PBS containing 5%, 10%, 20% and 50% glycerine at 4°C for 15 minutes, 1 hour, 10 hours, and 1 hour, respectively. Specimens were frozen in liquid nitrogen and sectioned with a Leica CM 1800 cryotome (4 - 6 µm). The extent of lesioned areas was determined after staining the aortic fragments with Oil Red O, or Nile Red and examined *en face*, with a Nikon Microphot-FXA fluorescence microscope with Hamamatsu Newvicon video camera.

Statistical analysis

Data were analyzed by one-way ANOVA. P values less than 0.05 were considered statistically significant. All data represent the mean values ± standard deviation.

Results

Changes in serum biochemical parameters - effect of amlodipine

Cholesterol

In the HH group, the serum cholesterol concentration increased steadily during the entire experimental period. As compared with control

animals, in which the cholesterol level had an average of 85 ± 12 mg/dl, after 14 weeks of hyperlipemic diet the level increased ~10 times (900 ± 50 mg/dl). Administration of Aml induced a slight decrease in serum cholesterol; however the decrease was not statistically significant (Fig. 1a).

Triglycerides

Triglycerides level increased in the HH group 6 times above the normal value (from 165 ± 64 mg/dl to 988 ± 485 mg/dl at week 14). In the HA group the decrease was not statistically significant (Fig. 1b).

TRAP values

TRAP values in the HH group decreased during the experimental period from 1056 ± 33 µmoles/l (week 0) to 741 ± 23 µmoles/l (week 14). Aml administration induced an increase in the HA group from 1057 ± 45 µmoles/l to 1158 ± 63 µmoles/l (week 14), which was statistically significant (Fig. 1c).

Pathological modifications in the lesion-prone areas of the hyperlipemic hamster

In general, development of atheroma included a sequence of events, generated by hypercholesterolemia and leading to fatty streak and occlusive fibrous plaque formation (Fig. 2). Ultrastructurally, these were identified as: endothelial cells (EC) activation, lipid accumulation in the intima, EC-monocyte interaction, monocytes migration into the intima and their activation as monocyte-derived macrophages, that take up lipids to become foam cells. This was followed by smooth muscle cells (SMC) migration from the media, their proliferation in the intima and enhanced synthesis of extracellular matrix, necrotic lipid rich core formation, calcium deposition, neovascularization, mural thrombi and occlusive acute thrombosis (Fig. 2).

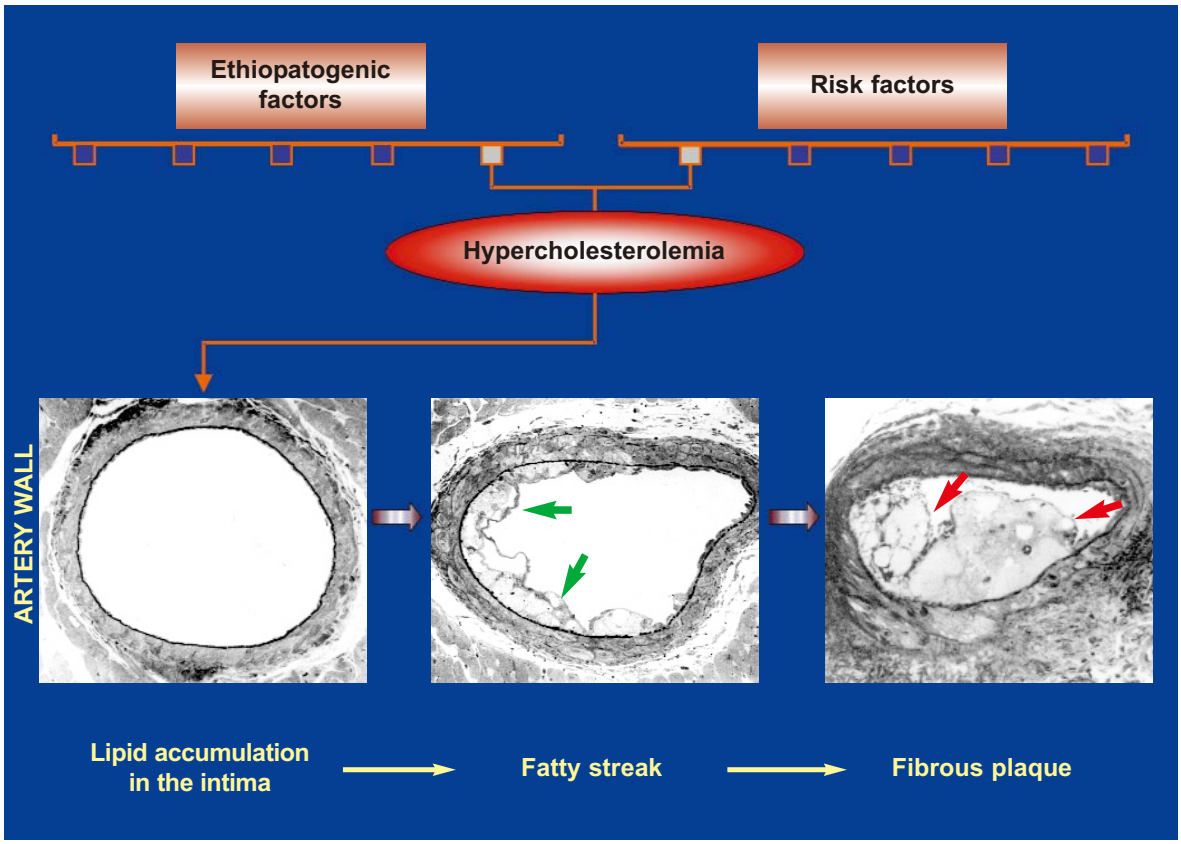

Fig. 2 Out of numerous ethiopathogenic and risk factors, hypercholesterolemia is sufficient to induce gradually occluding, fibrous plaques in coronary arteries of hyperlipemic hamsters.

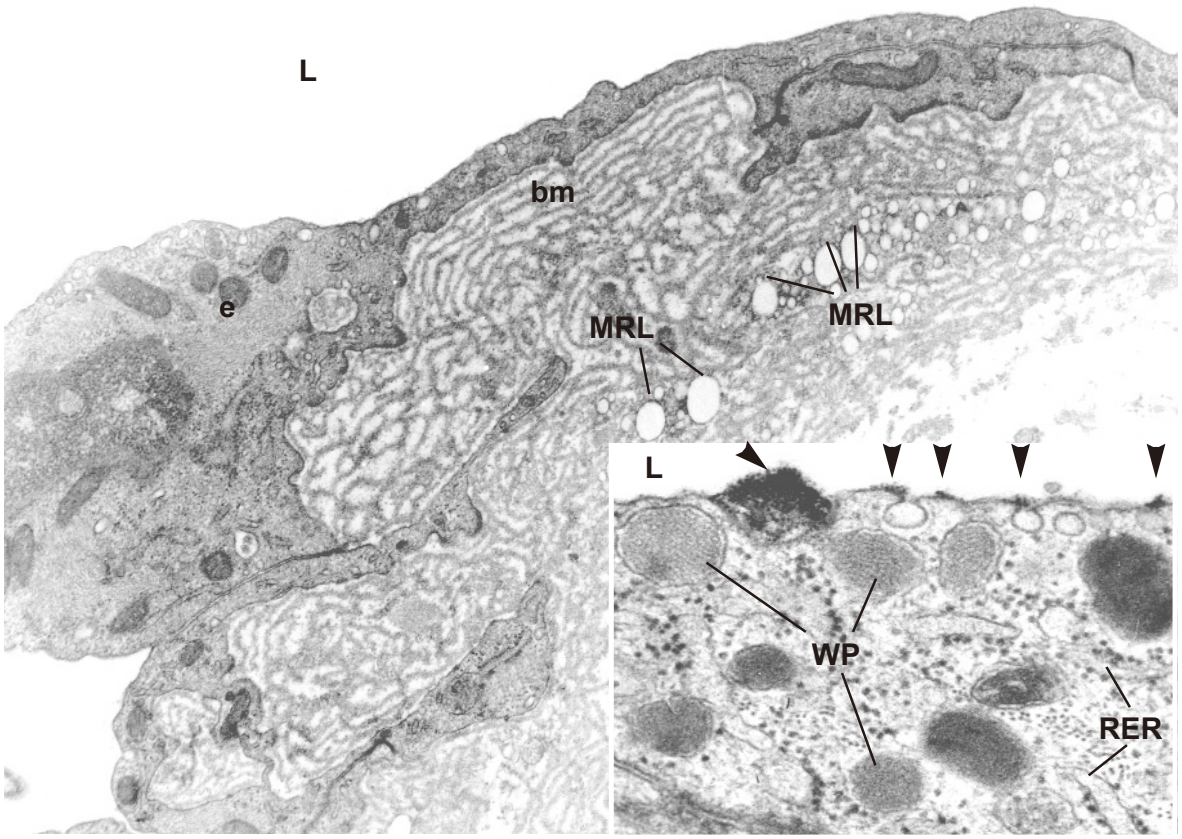

Fig. 3 Structural modifications of an aortic valve from a 4 week hyperlipemic hamster. Under the endothelial cells (e) there is a characteristic hyperplasic, multilayered basement membrane (bm), where numerous modified and reassembled lipoproteins (MRL) are trapped. x 20,000. Inset: the endothelium exibits a small number of decorated anionic sites on the plasmalemma, an aggregate of CF in a coated pit (arrows), and an increased number of biosynthetic organelles, such as rough endoplasmic reticulum (RER) and Weibel-Palade bodies (WP). L: vascular lumen. x 15,000.

Aortic valves

Aortic valves were the first affected segment of the vascular tree, prone to develop atherosclerotic lesions [18, 19]. Initially, two major changes related to EC function occured: increase in permeability for plasma proteins and enhanced biosynthetic activity. Two weeks of hyperlipemia induced increased transport of plasma lipoproteins and their accumulation in the subendothelium, within the meshwork of markedly hyperplasic, multilayered basal lamina (Fig. 3). The latter was probably the result of the increased synthetic activity of EC, expressed by the multiple copies of biosynthetic organelles, rough endoplasmic reticulum, Golgi complex, Weibel-Palade bodies (Fig. 3), microfilaments and microtubules. Within the subendothelium, lipoproteins appeared as uni- and multilamellar vesicles of various dimensions and electron-opacity (Fig. 3). These structures were similar to the modified and reassembled lipoproteins (MRL) detected within the sub-endothelial space of the aortic valves, aorta, coronary arteries of HH and man [15, 18-23]. The next event was the adherence to EC and diapedesis of blood monocytes (Fig. 4a), in areas rich in MRL, that were further identified as having

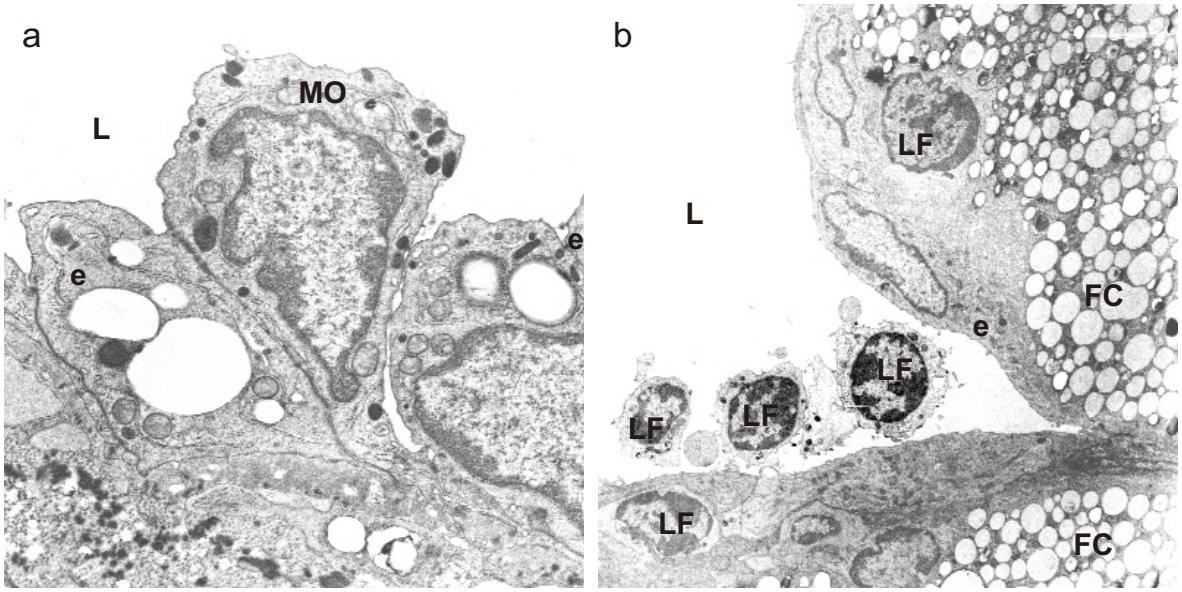

Fig. 4 A segment of the intima of an aortic valve from a hyperlipemic hamster. (**a**) circulating monocyte (MO) infiltrating between two endothelial cells (e), containing lipid droplets, in an area rich in MRL. x 10,000; (**b**) in an area rich in macrophage-derived foam cells (FC), lymphocytes (LF) are found either adherent to the endothelium (e) or within the subendothelium, next to FC. L: vascular lumen. x 5,000.

chemoattractant capacity. As blood cholesterol and peroxides level increased, the subendothelial macrophages were activated (expressing scavenger receptors SR-A and CD36), took up MRLs to become macrophage-derived foam cells (Fig. 4b), often forming cholesterol crystals. In time, lipid droplets accumulated into endothelial cells and valvular interstitial cells (Fig. 4a, b). Fatty streaks progressed rapidly, bearing large calcification areas in an expanded stroma with necrotic zones, and developed characteristic lacunae around the interstitial cells. As a late complication of the atheroma, lymphocytes adhered to the endothelium, and accumulated in the intima, contributing to the ongoing inflammatory process (Fig. 4b).

The net electrical charge of the endothelial surface explored with CF, perfused in situ, changed from the uniform labeling of the plasmalemma in normal EC to a patchy decoration, having large areas devoid of CF. After 14 weeks of hyperlipemia, CF was only scarcely found; coated pits and the neck of plasmalemmal vesicles were intensely decorated (inset, Fig. 3), as well as some enlarged or open junctions (not shown).

Coronary arteries

As in the aortic valves, in coronary arteries the endothelium exhibited a secretory phenotype and numerous MRLs of various sizes were present within an expanded, reticulated basal lamina. SMC, migrated into the intima, switched from the contractile to a secretory phenotype, displaying multiple copies of biosynthetic organelles (Fig. 5a), responsible for the hyperplastic basal membrane (Fig. 5b). The expanded extracellular matrix that surrounded SMCs contained calcification centers, that evolved into large calcification cores, occupying most of the coronary wall. Later, SMCs endowed with active scavenger receptors, took up lipids by a non-saturable process and became foam cells (Fig. 5c).

Aortic arch

The sequence of events was similar to that described for coronary arteries, except that macrophage-derived foam cells were more numerous than SMC.

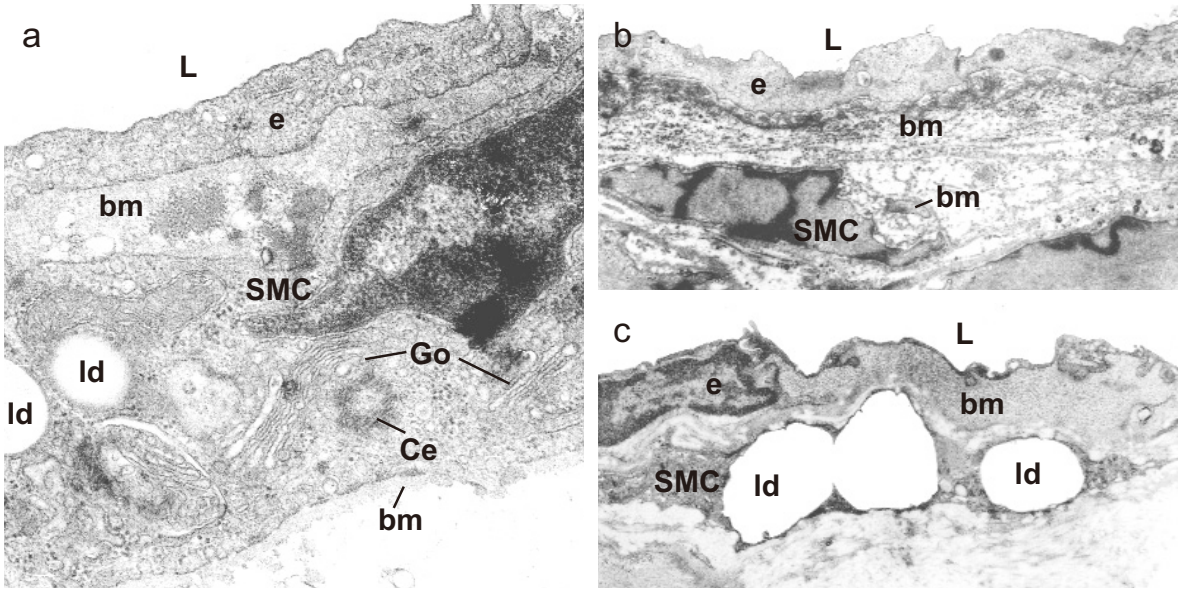

Fig. 5 Smooth muscle cell (SMC) migrated in the intima of a coronary artery of a hyperlipemic hamster switched to a secretory phenotype. (**a**) Numerous Golgi apparata (Go), lipid droplets (ld) and centrioles (Ce), the latter suggesting that SMC are actively dividing, x 30,000; (**b**) Intimal SMC surrounded by a highly proliferated multilayered basement membrane (bm) and a hyperplasic extracellular matrix, x 8,000; (**c**) with the progression of hyperlipemia, lipid droplets (ld) accumulate intracellularly and convert SMC into foam cells. e: endothelium, L: lumen. x 10,000.

Effect of amlodipine on the uptake and transcytosis of LDL in the arterial wall

Since increased transport of LDL was a common occurrence in HH, we followed in vivo uptake of LDL coupled to DiI in lesion-prone areas and the effect of Aml in this process. After 24h in the circulation, the transcytosed fluorescent conjugate accumulated in fatty streaks of the aortic arch (Fig. 6b) and sigmoid valves (Fig. 7a) of HH, and was undetectable in the same areas of HN (Fig. 6a). The fibrous cap of the atheroma had a reduced uptake, foam cells and lymphocytes were unmarked, as was the necrotic core (not shown). Amlodipine treated hamsters (HA) developed considerably fewer and smaller lesions, as compared to HH, and were mostly found in aortic valves. On cryosections of the aortic arch, LDL-DiI accumulation was hardly visible (Fig. 6c). In the aortic valves, after 6 weeks of Aml administration, the numerous macrophages present at the valve insertion took up LDL-DiI (Fig. 7b). After 12 weeks of treatment, the uptake diminished, as evidenced by the absence of the red fluorochrome, indicating the scarcity of transcytosed LDL (Fig. 7c).

Discussion

Hamsters have been used for the evaluation of numerous pharmacologic agents with varying mechanisms of action. Testing the inhibitors of CCB [24, 25], inhibitors of angiotensin converting enzyme, such as captopril and fosinopril [26], and the ACAT inhibitor octimibate [27] have shown that hamster is a useful model for assessing the effects of many drugs.

Studies from literature showed that amlodipine significantly lowers the intracellular cholesterol content of smooth muscle cells derived from atherosclerotic plaques [28] and restores the cholesterol enriched membrane bilayer to normal [29].

In our experiments, Aml was ineffective in lowering total serum cholesterol and triglycerides level, as was reported in humans [30]. It is worth mentioning that in HN the standard deviation for both cholesterol and triglycerides was lower than in HH and HA, suggesting the large variability in the animal respons to the hyperlipemic diet and Aml administration. However, our results demonstrated that Aml exerts an anti-atherosclerotic protection by: (i) acting

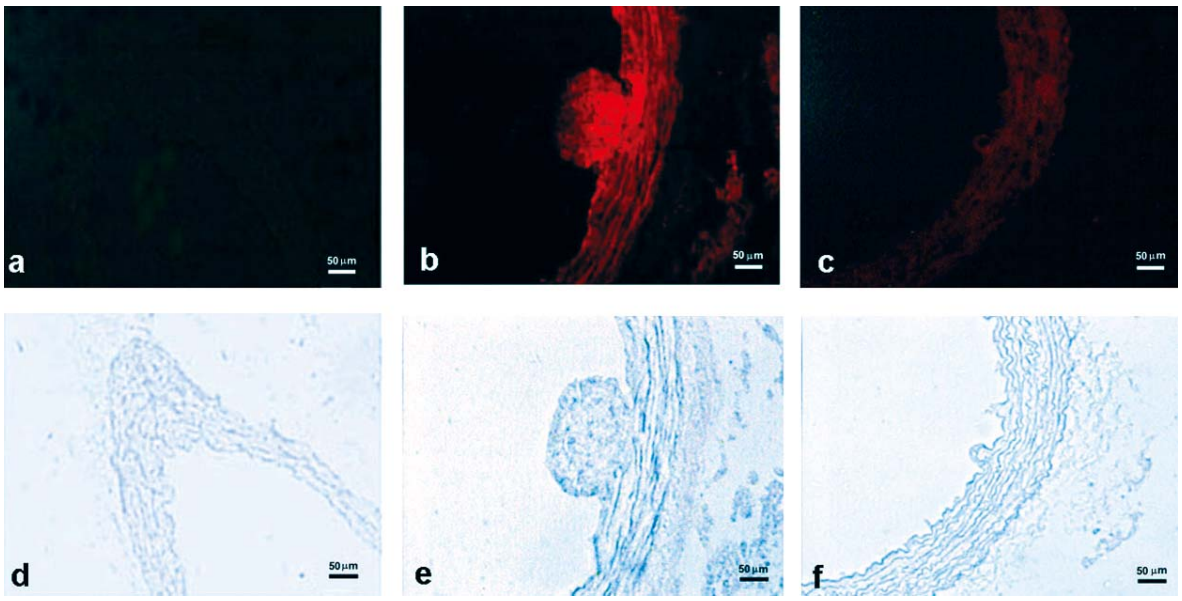

Fig. 6 Cryosections of hamsters aortic arch, 24h after the injection in vivo of LDL-DiI: (a) absence of red fluorochrome in normal aorta, (b) uptake of LDL-DiI in a large fatty streak of a 12 week hyperlipemic hamster; note intense fluorescence of the macrophage-derived foam cells, (c) Amlodipine treatment for 12 weeks reduces the fluorescence to background level; (d-f) light microscopy images of the same sections.

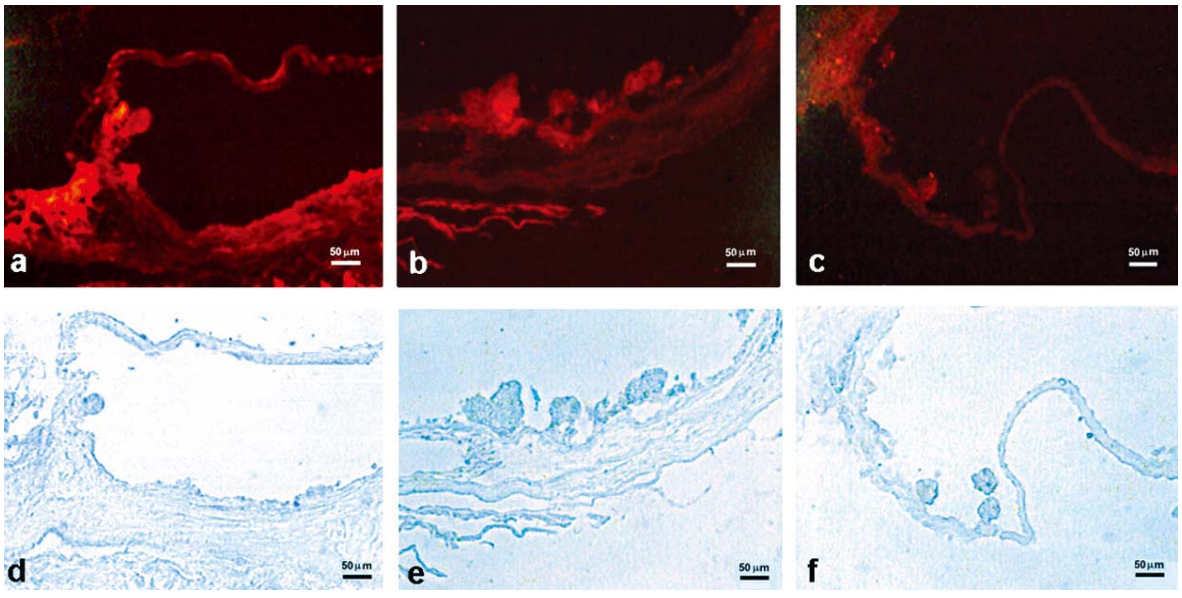

Fig. 7 Cryosections of aortic valves from hamsters injected *in vivo* with LDL-DiI: (a) uptake of LDL evidenced by the red fluorochrome (DiI) in a 12 week hyperlipemic hamster; (b-c) diminished uptake of LDL in a hyperlipemic hamster treated with amlodipine for 6 weeks (b) and 12 weeks (c); (d-f) light microscopy images corresponding to above sections.

as an antioxidant agent, (ii) diminishing the uptake of LDL by the vessel wall, and consequently (iii) reducing the extent and size of lesions.

Based on the similarities between HH and humans in the lipoprotein metabolism and in the sequence of vascular events leading to atherosclerotic plaque formation, the hamster model can be reliably used for testing the effects of drugs on serum lipids and proteins, as well as on the cellular components of the vessel wall.

Acknowledgements

The authors are grateful to Loredan Niculescu, PhD student, for the determination of TRAP values, to Ioana Andreescu for the excellent technical assistance, and Laura Vladimirescu, MD, for the attentive care of the hamsters in the animal housing department. Work supported by grants from the Romanian Academy, Ministry of Education and Research, and National Institues of Health, USA.

References

1. **Stary H.C.,** The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life, *Eur. Heart J.*, **11**: 3-19, 1990
2. **Velican C., Velican D.,** Arterele coronare in cardiopatia ischemica. Editura Medicala/ Romania, Bucuresti, 1992
3. **Sima A., Bulla A., Simionescu N.,** Experimental obstructive coronary atherosclerosis in the hyperlipidemic hamster, *J. Submicrosc. Cytol. Pathol.,* **22**: 1-16, 1990
4. **Sullivan M.P., Cerda J.J., Robbins F.L., Burgin C.W., Beatty R.J.,** The gerbil, hamster, and guinea pig as rodent models for hyperlipidemia, *Lab. Anim. Sci.,* **43**: 575-78, 1993
5. **Bishop R. W.,** Structure of the hamster LDL receptor gene, *J. Lipid Res.*, **33**: 549-57, 1992
6. **Chen L., Haught W.H., Yang B., Saldeen T.G.P., Parathasarathy S., Metha J.,** Preservation of endogenous antioxidant activity and inhibition of lipid peroxidation as common mechanisms of antiatherosclerotic effects of vitamin E, lovastatin and amlodipine, *J. Am. Coll. Cardiol*., **30**: 569-75, 1997
7. **Digiesi V., Fiorillo C., Cosmi L., Rossetti M., Lenuzza M., Guidi D., Pace S., Rizzuti G., Nassi P.,** Reactive oxigen species and antioxidant status in essential arterial hypertension during terapy with dihydropiridine calcium channels antagonist, *Clin. Ther*., **151**: 15-18, 2000
8. **Tardif J.C.,** Insights into oxidative stress and atherosclerosis, *Can. J. Cardiol.*, **16**: 2D-4, 2000
9. **Steinberg D.,** Low density lipoprotein oxidation and its pathobiological significance, *J. Biol. Chem.,* **272**: 20963-66, 1997
10. **Esterbauer H., Schmidt R., Hayn M.,** Relationships among oxidation of low-density lipoprotein, antioxidant protection, and atherosclerosis, *Advance in Pharmacology*, **38**: 425-56, 1997
11. **Holvoet P., VanCleemput J., Collen D., Vanhaecke J.,** Oxidized low density lipoprotein is a prognostic marker of transplant- associated coronary artery disease, *Arterioscler. Thromb. Vasc. Biol.,* **20**: 698-702, 2000
12. **Fukumoto M., Shoji T., Emoto M., Kawagishi T., Okuno Y., Nishizawa Y.,** Antibodies against oxidized LDL and carotid artery intima-media thickness in a healthy population, *Arterioscler. Thromb. Vasc. Biol.,* **20**: 703-707, 2000
13. **Jones N.L., Reagan J.W., Willingham M.C.,** The pathogenesis of foam cell formation- Modified LDL stimulates uptake of co-incubated LDL via macropinocytosis, *Arterioscler. Thromb. Vasc. Biol.,* **20**: 773-81, 2000
14. **Mehrabi M. R., Sinzinger H., Ekmekcioglu C., Tamaddon F., Plesch K., Glogar H.D., Maurer G., Stefenelli T., Lang I.M.,** Accumulation of oxidized LDL in human semilunar valves correlates with coronary atherosclerosis, *Cardiovasc. Res.,* **45**: 874-882, 2000
15. **Simionescu N., Sima A., Dobrian A., Tirziu D., Simionescu M.,** Pathobiochemical changes of the arterial wall at the inception of atherosclerosis. In: Vollmer, Roessner (eds.), Current Topics in Pathology, Berlin, Springer Verlag, 1993, pp. 1-45
16. **Valkonen M., Kuusi T.,** Spectrophotometric assay for total peroxyl radical-trapping antioxidant potential in human serum. *J. Lipid. Res*. **28**: 823-833, 1997
17. **Innerarity T. L., Pitas R. E., Mahley R. W.,** Lipoprotein – Receptor Interactions. In: Albers J. J., Segrest J.P., eds. Methods in Enzymology, vol 129, Academic Press, Inc./USA, NY, 1986, pp. 542-546.
18. **Filip D.A., Nistor A., Bulla A, Radu A., Lupu F., Simionescu M.,** Cellular events in the development of valvular atherosclerosis lesions induced by experimental hypercholesterolemia. *Atherosclerosis*, **67**: 199-214, 1987
19. **Nistor A., Bulla A., Filip D.A., Radu A.,** The hyperlipidemic hamster as a model of experimental atherosclerosis. *Atherosclerosis,* **68**: 159-173, 1987
20. **Sima A., Popov D., Starodub O., Stancu C., Cristea C., Stern D., Simionescu M.,** Pathobiology of the heart in experimental diabetes: immunolocalization of LDL, IgG and AGE-proteins in diabetic and/or hyperlipidemic hamster. *Lab. Invest.*, **77:** 3-18, 1997

21. **Simionescu M., Simionescu N.,** Modulations and dysfunctions in the artery intima during the prelesional stage of hypercholesterolemic atherogenesis. In: New Horizons in Coronary Heart Disease. Born G.V.R., Schwartz C.J. (eds.), Current Science Ltd., London, pp. 11.1-11.14, 1993
22. **Simionescu N., Vasile E., Lupu F., Popescu G., Simionescu M.,** Prelesional events in atherogenesis: Accumulation of extracellular cholesterol-rich liposomes in the intima and cardiac valves of the hyperlipidemic rabbit. *Am. J. Pathol*., **123**: 109-125, 1986
23. **Tirziu D., Dobrian A., Tasca C., Simionescu M., Simionescu N.**, Intimal thickening of human aorta contain modified reassembled lipoproteins, *Atherosclerosis*, **112:**101-114, 1995
24. **Raicu M., Sima A., Toporan D., Stancu C., Protopopescu T., Ioan Al., Simionescu M.,** Nifedipine induces a pro-atherogenic effect on smooth muscle cells in culture, incubated with LDL isolated from patients with coronary diseases, *Eur.Heart J.*, **20 :** 66, 1999
25. **Raicu M., Pojoga L., Simionescu N., Simionescu M.,** Differential effect of two calcium channel blockers - nifedipine and diltiazem - in atherogenesis in hypercholesterolemic hamster, *J. Submicrosc. Cytol. Pathol.,* **28 :** 265-75, 1996
26. **Kowala M.C., Recce R., Beyer S., Aberg G.,** Regression of early atherosclerosis in hyperlipidemic hamsters induced by fosinopril and captopril, *J. Cardiovasc. Pharmacol*., **25:** 179-86, 1995
27. **Kowala M.C., Mazzucco C.E., Hartl K.S., Seiler S.M., Warr G.A., Abid S., Growe R.I.,** Prostacyclin agonists reduce early atherosclerosis in hyperlipidemic hamsters, *Arteriosclerosis and Thromb.,* **13:** 435-44, 1993
28. **Orekhov A.N., Tertov V.V., Pivovarova E.M.,** The effect of antihypertensive agents on atherosclerosis-related parameters of human aorta intimal cells, *Cardiology*, **89**: 111-18, 1998
29. **Tulenko T.N., Sumner A.E., Chen M., Huang Y., Laury-Kleintop L., Ferdinand F.D.,** The smooth muscle cell membrane during atherogenesis: a potential target for amlodipine in atheroprotection, *Am. Heart J*., **141**: S1-11, 2001
30. **Kramsch D.M.,** Limits of lipid-lowering therapy: the potential benefits of amlodipine as an antiatherosclerotic agent, *Int. J. Cardiol*., **62**: S119-24, 1997