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ASPECTS OF THE AMLODIPINE PLEIOTROPY IN BIOCHEMISTRY, PHARMACOLOGY AND CLINICS

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ABSTRACT

Amlodipine is the third generation calcium antagonist, 1,4-dihydropyridine derivative with the prolonged duration of the antihypertensive action, especially blocking L-type Ca^{2+} ion channels. It promotes beneficial therapeutic effect by coronary and other blood vessel diseases and thus delays development of the atherosclerosis. It has several known trade names, the most mentioned is *Norvasc*. Amlodipine is well tolerated in the clinics, it could be used in combinations with other drugs – diuretics, angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, statins. Amlodipine at nanomolar concentrations binds to the voltage-dependent L-type calcium channels. It possesses optimal lipophylicity. Amlodipine also influences the NO-dependent metabolic processes, stimulates NO synthesis and prolongs NO action duration. Results of the studies of the amlodipine pharmacological and clinical properties are summarized in several reviews. The present review contains opinion from the scientific works of the last decades about the multisided or pleiotropic amlodipine mechanisms of action, it contains information about sometimes controversial clinical studies of the amlodipine vaso- and cardioprotective activity.

INTRODUCTION: Amlodipine is the third generation calcium antagonist, 1, 4-dihydropyridine (1, 4-DHP) compound with prolonged action. It has expressed properties of Ca^{2+} channel (especially L-type and T-type) blockers (CCB). Besides, it exerts multisided (pleiotropic, namely, when a drug has actions other than those for which the compound was specifically indicated or developed) effects on other metabolic processes. It is the basis for the beneficial therapeutic action at heart and vasculature diseases and delays the development of atherosclerosis [1 - 11]. This drug is well tolerated by patients. It has no significant side effects. It could be used in the monotherapy, as well in combination with other drugs-diuretics, angiotensine-converting enzyme (ACE) inhibitors, antagonists of the angiotensine II receptors, statins [12, 13, 14, 15].

Amlodipine at nanomolar concentrations binds to the voltage-dependent L-type calcium channels. It has optimal lipophylicity, and it has a free aminogroup, which garantees the good bioavailability [9]. Amlodipine also influences the NO-dependent metabolic processes too, it stimulates NO synthesis and prolongs NO action duration [16].

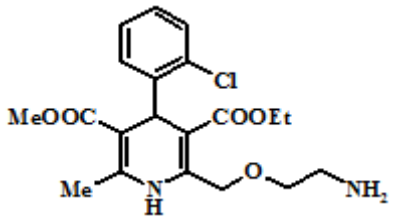
Results of the studies of amlodipine pharmacological and clinical properties are summarized in several reviews [9, 12, 17 - 20].

This review summarizes outlook about experimental materials from the last decades concerning amlodipine pleiotropy in the action mechanism, as well sometimes controversial clinical observations about its cardio-protective and vazoprotective action.

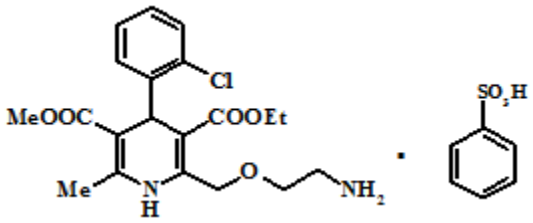
Amlodipine overall characteristics: Amlodipine *(Amlodipinum*, Drugbank ID: DB00381, http://www.drugbank. ca/drugs/DB00381 [21], $C_{23}H_{27}N$; http://en.wikipedia.org/wiki/Amlodipine [22]), is the third generation dihydropyridine type chiral compound, calcium antagonist with expressed lipophilicity (lgP ~1.90 (exp.), 2.22 (theor.), according other data: 3.00 (exp.), 3.30 (theor.)) currently available in the market and used in the medicinal praxis as a racemate [1:1 mixture of (*R*)-(+)- and (*S*)-(–)-amlodipine], its chemical (IUPAC) name is: (*RS*)-3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chloro-phenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarbo-xylate).

Ca antagonist activity differs for both its enantiomers: S(-)-enantiomer is ~1000 times more active as R(+)-enantiomer. As R(+)-enantiomer is responsible for NO release, the summary amlodipine therapeutic efficacy partially is formed also by R(+)-enantiomer. S(-)-enantiomer completely lacks NO releasing activity.

Amlodipine and *Norvasc* (amlodipine besylate) structural formulae:



AMLODIPINE



NORVASC (AMLODIPINE BESYLATE)

Amlodipine is a generic (*Pfizer* patent expired at 2007) drug, and is known with several authorized trade names (about 446 brands, including 24 of that for S-Amlodipine): *Agen* (Zentiva - Czech Republic), Aken (Kendrick Farmaceutica - Mexico), Amcard (Apex Pharma Ltd. - Bangladesh), Amdepin (Cadila Pharmaceuticals - India), Amdipin (Laboratorios Lafrancol - Colombia), *Amlodine* (*Westfield Pharmaceuticals, a division of InnoGen - Philippines), Amlodac, Amlodis, Amlogard, Amlopine (Berlin (Thailand) Pharmaceutical Industry Co Ltd. - Thailand*), *Amlopres*, *Amtas*, *Amvaz*, *Coroval*, *Dailyvasc* (*Xeno* Pharmaceuticals), Istin(United Kingdom, Ireland), Klodip, Lipinox, Lopin (Edruc Ltd. - Bangladesh), Lotrel, Norvasc (Pfizer - North America and in some European countries), Norvasc and Perivasc (Australia) [http://en.wikipedia.org/wiki/Amlodipine].

In the combined therapy amlodipine is combined either with ACE inhibitors -lisinopril (Hipril, containing 5mg from each of the both drugs, Micro Labs - India), benazepril, captopril, perindopril, or beta-blockers - nebivolol hydrochloride a.o., alfa-1 adrenoreceptor antagonist *Terazosin*, statins-simvastatin, atorvastatin, valsartan, telmisartan, a.o. (see http://drugs-about.com/ing/amlodipine.html).

Drugstores share many drugs, containing amlodipine (or its besylate, or mesylate, or camsilate, or maleate, or succinate salt forms) (in 3 strengths - 2.5 mg, or 5.0 mg, or 10.0 mg) as the only one single (solely) active substance - *Norvasc* (Pfizer - North America and in some European countries), *Agen* (*Zentiva*), *Amlocard* (*Hexal*), *Lofral* (*Mepha*), *Tenox* 5mg (*KRKA*) (it should not to be mixed with another drug of the same name *Tenox* (containing as the active substance *Temazepam*)), *Amlodipine Medochemie* (*Medochemie*), *Amlodigamma 5 mg* tablets (*Worwag Pharma*); *Amlopress* (*Cipla*), *Amdepin* (*Cadila*) (India); *Amlodipin Olainfarm 5mg* tablets and *Amlodipin Olainfarm 10mg* (Amlodipinum) tablets (Latvia).

In accordance with the international guidelines and therapeutic schemes '*ESC/ESH 2007 Guidelines for the management of arterial hypertension'*, amlodipine is one of the drugs of choise for the prevention and cure of elevated arterial blood tension, stenocardia and other cardiovascular diseases as the monotherapeutics or in the combination with other antihypertensives or antianginal drugs [See http://eurheartj.oxfordjournals.org/content/28/12/1462.full.pdf [23].

Amlodipine is a 1, 4-dihydropyridine type calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the transmembrane influx of calcium ions into the cells of vascular smooth muscle and cardiac muscle through outer membranes of their cells [1, 7, 8], with a greater effect on vascular smooth muscle cells than on cardiac muscle cells. The contractile processes of cardiac muscle and vascular

smooth muscle are dependent on the concentration of calcium ions in cytoplasm. Amlodipine has no influence on serum concentration of Ca^{2+} ions.

Within the physiologic pH range, amlodipine is an ionized compound (pK_a=8.6), and its kinetic interaction with the calcium channel receptor is characterized by a gradual rate of association and dissociation with the receptor binding site, resulting in a gradual onset of effect.

Experimental data suggest that amlodipine binds to both dihydropyridine and non-dihydropyridine binding sites.

Amlodipine is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure.

The precise mechanisms by which amlodipine relieves angina have not been fully delineated, but are thought to include the following:

Exertional angina: In patients with exertional angina, amlodipine reduces the total peripheral resistance (after-load) against which the heart works. The unloading of the heart is thought to decrease ischemia and relieve effort angina by reducing myocardial energy oxygen consumption and oxygen requirements, at any given level of exercise.

Vasospastic angina: Amlodipine has been demonstrated to block the constriction in response to the elevated levels of each calcium, or potassium, or epinephrine, or serotonin, and or thromboxane A_2 analog, and restore blood flow in coronary arteries and arterioles in experimental animal models and in human coronary vessels (*in vitro*) **[9, 22, 24]**. This inhibition of coronary spasm is responsible for the effectiveness of amlodipine in vasospastic (Prinzmetal's or variant) angina. Amlodipine and amlodipine besylate are both calcium blockers, however the former works better with both angina and hypertension while the latter works on hypertension alone.

Preclinical pharmacological investigations: Amlodipine causes stepvise dose-dependent hypotensive effect in the animal experiments, without occurrence of the reflector tachycardy. The maximal decrease of blood presuure is observed in the 8-th hour after the peroral amlodipine application, the hypotensive action duration is about 24 hours [7]. Prolonged (30 weeks) introduction of amlodipine in SHR rats retards the increase of arterial blood pressure and development of the myocardial hypertrophy [8].

Antianginal and antiischemic amlodipine action (by decrease of calcium overload and endothelial dysfunction) is shown in the experiments with dogs [25]: the decrease of the infarct zone is observed [26], left ventricule function was not influenced [27]. In the experiments on the myocard infarct model (in rats treated with isoproterenol) amlodipine almost normalized tissue protection system (revealing antioxidant properties) [14].

a. **The role of amlodipine and kinins in the NO production and release and its implications for the treatment of CVD:** *In vivo*, amlodipine induced coronary vasodilatation, influencing both adenosine- and NO-dependent mechanisms [28]. The vasodilatation was reduced through inhibition of NO synthesis (with use of a substituted arginine molecule), and amlodipine has been shown to increase peripheral and coronary blood flow [1]. Experimental studies of amlodipine *in vitro* (pigs coronar vessels, pigs endothelial cell culture) revealed increase of the bioavailability of the endothelium to NO, firstly – by increasing NO production, secondly – due to the antioxidant properties of amlodipine, thus prolonging NO action [16].

   Amlodipine (10 micromoles / kg) decreased the resistance of the femoral artery (in vivo perfusion of rabbit femoral artery) and increased NO concentration in the femoral vein (verapamil and nifedipine did not possess such effect, despite these drugs too decreased the resistance of the femoral artery). These amlodipine effects weakened bradykinin B2 receptor antagonist HOE 140, 30 mg / kg [29].

   Authors concluded, that vasorelaxating activity of amlodipine partly is NO-dependent, because it was weakened by blockage of NO-synthase (with N(G)-nitro-L-arginine methylester, L-NAME, 1 micromole /kg), however in the presence of verapamil and

nifedipine the vasorelaxation was not changed-thus not connected with NO in these experiments (with rabbit femoral artery *in vivo*). Amlodipine action additionally could be connected with B2 receptor activation and local rise of bradykinin, because, as mentioned, bradykinin B2 receptor antagonist HOE 140 diminished NO increase in the venae caused by amlodipine.

In vitro, amlodipine, unlike nifedipine or diltiazem, caused a dose-dependent release of nitrite, the hydration product of NO. The release of nitrite or inhibition of tissue oxygen consumption was similar in magnitude to those caused by the 3 ACE inhibitors captopril, enalapril, and ramiprilat. Amlodipine effectively blocks serum ACE activity *in vi*tro in the concentrations near to enalapril [29]. Amlodipine not only reduces the resistance of the smooth muscles of the vessels, but relaxes spontaneous contractions of the uterus (*in vitro*) [30]**.**

The calcium channel blocker amlodipine promotes the unclamping of eNOS from caveolin in endothelial cells [31]. There are no L-type calcium channels on endothelial cells. As amlodipine activates eNOS, there must be a mechanism independent of its CCB properties whereby amlodipine releases NO [1]. Amlodipine enhanced endothelial NO production through a bradykinin-dependent pathway. This ability of amlodipine to stimulate NO synthesis was dose dependent and could not be reproduced by other CCBs (diltiazem, nifedipine), but could be replicated by the ACE inhibitor enalapril [1].

Amlodipine specifically increased the intracellular concentrations of endothelial nitric oxide synthase protein, leading to an enhanced rate of NO production. The molecular mechanism for progression of congestive heart failure (CHF) may involve cytokine overexpression.

An adverse event-CHF or death-occurred more commonly in patients with higher IL-6 levels. Amlodipine lowers plasma IL-6 levels in patients with CHF. The beneficial effect of amlodipine in CHF may be due to a reduction of cytokines such as IL-6 [32]. Reduction in NO production or the biologic activity of NO contributes to the development of heart failure, diabetes, atherosclerosis, and hypertension. In states where eNOS is down-regulated, the activity of the enzyme can be enhanced, and drugs such as amlodipine, other CCBs, and ACE inhibitors might confer benefit by restoring NO production.

NO-dependent regulation of the oxygen consumption in explanted human hearts were greater when amlodipine was combined with an ACE inhibitor. The combination was found to be more than additive, indicating a true synergism [1]. This can be explained through an understanding of the role of kinins in the mechanism of action of both drugs. ACE inhibitors block the breakdown of kinins, and amlodipine stimulates kinin formation by activating or releasing kallikrein [1, 12].

b. **Inhibition of cytokine- and free-radical induced endothelial apoptosis:** Tumour necrosis factor-α (TNF-α) is a cytokine that is elevated in atherosclerosis and mediates damage to the vessel wall during the inflammatory process. Cytokines such as TNF-α can activate resident macrophages that interfere with the structural integrity of the fibrous cap. Disruption of the heterogeneous fibrous plaque reveals the underlying, highly thrombotic lipid core, leading to acute coronary syndromes in patients with coronary artery disease (CAD). In addition to its effect on plaque destabilisation, TNF-α also interferes with endothelial function, leading to the expression of proteins that mediate programmed cell death or apoptosis. Amlodipine has been shown to inhibit TNF-α-induced endothelial apoptosis in a dose-dependent manner, starting at low nanomolar levels (10.0 nM) [1].

c. **Amlodipine anti-atherogenic action and its mechanisms:** In the atherosclerosis animal (rats, rabbits, monkeys, fed with cholesterol-rich food) models amlodipine dose-dependently diminishes the atherosclerotic lesions of vessels [9, 11, 17] and cause atheroprotective effect, diminishing the accumulation of the cholesterol into the aorta wall and decreasing lipid peroxidation of the aorta, increasing superoxide dismutase (SOD) activity in the blood and aorta tissue (in the experiments with rabbits, fed with cholesterol, if additionally

amlodipine 5 mg/kg/day per os were used), retarding the consumption of the endogenous vitamin E. This effect may be connected with the amlodipine antioxidant properties [33].

Recent clinical trials also revealed amlodipine antiatherogenic action, mechanism however is not fully understood. In the experiments with the hypertensive SHR stroke-prone rats the observations were made that amlodipine remarkably influenced endothelial NO synthase and proteinkinase cascade, which could be the basis for the vascular protection [34]. The ability of amlodipine to retard the development of the atherosclerotic lessions was observed, as well as the regress of atherosclerosis. This is probably connected with the decrease of the oxidative stress and inflammation (in the experiments with mice deficient to apolipoprotein-E – ApoEKO, the amlodipine dose – 3 mg/kg/per day, 10 weeks) [35].

Combining amlodipine (dose - 0.003% (w/w) with food, which is equivalent to 3.5 mg amlodipine / kg living mass/ per day) together with atorvastatin (dose - 0.0035% (w/w) atorvastatine with food, which is equivalent to 4 mg/ kg living mass/ per day) the remarkable increase of the antiatherosclerotic effect was observed [36], as well as decreased mineralization of the atherosclerotic plaques (experiments with APOE*3 Leiden transgenic mice) [37]. Atheroprotective amlodipine action partly was observed as the result of the inhibition of glycation end products (AGE) accumulation [38] (see also subpart **I**).

Medication of the experimental animals (kept on atherogenic diet) with amlodipine during 8 weeks decreased total cholesterol, low density lipoprotein cholesterol (LDL), triglyceride (TG) and endothelin level in the plasma and aortic tissue [39].

Amlodipine in 1-10 mkM concentrations (especially in 35 mkM concentration, remarkably exceeding the toxicity level –10 mkM) and other CCB influenced the synthesis of proteoglycans (glucosamineglycans, GAG) in blood vessel walls [40]: decreased it molmass and charge density. These events influenced the connection of proteoglycans with LDL. In this aspect both stereoisomers of amlodipine are active. In the therapeutic concentrations this effect is less pronounced. Modulation of GAG synthesis is mentioned as one of the initiating factors in the atherosclerosis development.

Amlodipine action is more prolonged as compared with nifedipine a.o. classical CCBs. Amlodipine is in the charged state at physiologic pH levels (protonated amino group). This positive charge leads to strong electrostatic interactions of amlodipine with membrane phospholipid head groups. Electrostatic interactions result in the drug's concentrations in the membrane at levels >10 000-fold higher than in the surrounding aqueous environment. There is a sustained reservoir of amlodipine available for binding over time to calcium channel receptors within the plasma membrane of vascular smooth muscle cells (VSMCs) [1, 12].

It explains the extended duration of the activity of amlodipine compared with shorter-acting, less lipophilic and less charged agents [1]**.** Additionally, the positive charge and strong lipid affinity of amlodipine enable it to inhibit aggregation of modified LDLs, a key step in foam cell formation mediated by the electronegative properties of oxidized lipid. This atheroprotective effect of amlodipine could not be reproduced by other antihypertensive agents (other CCBs, ACE inhibitors) that lack such physical chemical characteristics.

In the experiments using the model of mouse obstructive coronary disease (M encephalomyo-carditis virus) amlodipine remarkably decreased histopathological parameters of the myocardial damage and increased life-span, in comparison with the control group. This therapeutic effect could be connected with inhibition of the overproduction of NO [41]. L-type CCBs indicated that these drugs were potent and selective vasodilators because of their ability to reduce intracellular SMC Ca^{2+} concentration. Increasing basic and clinical data suggest that there are non–calcium-related pleiotropic actions of CCBs.

Amlodipine can regulate membrane fluidity and cholesterol deposition, stimulate NO production to recruit its biologic actions, act as an antioxidant, and regulate matrix deposition.

The PREVENT study showed significant clinical benefits of amlodipine therapy, compared with placebo, including a marked reduction in cardiovascular morbidity and significant slowing in the progression of carotid atherosclerosis. These findings support a potentially new therapeutic role for certain third-generation dihydropyridine-type CCBs, such as amlodipine, in the treatment of atherosclerosis. However other authors have reported lack of amlodipine antiatherosclerotic properties [42], namely in diabetic animals [43].

d. **Amlodipine renoprotective action in the case of the experimental diabetic nephropathy:** Amlodipine decreased albuminuria, pathological lessions and accumulation of proteins of the extracellular matrix in the kidneys in experimental diabetic rats with diabetic nephropathy [44, 45].

e. **Amlodipine as an platelet aggregation inhibitor:** It is shown that amlodipine inhibits platelet aggregation [4], which probably is caused by stimulation of NO, because NO is natural inhibitor of the thrombocyte aggregation [46]. Also other experiments confirm the ability of amlodipine to modulate NO production in the endothelium [47, 48, 49]. (See also subpart **a**)

f. **Antioxidant properties of amlodipine:** The charged 1, 4-dihydropyridine calcium antagonist amlodipine had the highest affinity (amlodipine > verapamil >> diltiazem) for the membrane bilayer ($Kp=10^4$) and produced the largest changes in membrane thermodynamic properties, including a reduction in the thermal phase transition temperature (-11%), enthalpy (-14%) and cooperative unit size (-59%), relative to control phosphatidylcholine liposomes [13].

Both *in vitro* and *in vivo* studies have shown that amlodipine inhibits oxidative damage of lipids associated with cellular membranes and lipoprotein particles (LDL) [12]. Under controlled experimental *in vitro* conditions (Total Oxyradical Scavenging Capacity (TOSC) assay), amlodipine revealed antioxidant and antiradical activity: inhibited lipid peroxide formation and trapped reactive oxygen species hydroxyl- and peroxyl-radicals (ROS scavenging capacity), at concentrations as low as 10.0 nM (which is remarkably less as for used as reference (classical) antioxidants – GSH, uric acid and Trolox), independent of the calcium channel modulation [50].

TOSC assay is based on the reaction of artificially generated oxyradicals with α-keto-γ-methiolbutyric acid (KMBA), which is completely oxidized to ethylene. In the presence of an antioxidant molecule, competition with KMBA ensues, resulting in a reduction of free radical generation, hence, lower ethylene production.

Amlodipine showed efficiency as scavenger of peroxyl radicals (TOSC: 5945 ± 544 units/mg) significantly higher (>50%, P <0.001) than that of GSH (2733 ± 636 units/mg), and 70% lower (P < 0.0001) than the value obtained with uric acid (18144 ± 696 units/mg) and trolox (17522 ± 734 units/mg). Of interest, the scavenging capacity of amlodipine towards hydroxyl radicals (1455 ± 154 units/mg) was 320% higher (P <0.00001) than that of GSH (358 ± 112 units/mg), 20% higher than that of uric acid (1198 ± 121 units/mg), and 100% higher than that of trolox (759 ± 143 units/mg).

Amlodipine increased enzyme activity of paraoxonase (PON) and glutathione peroxidase (GSH-Px), however decreased glutathione reductase (GSSG-R) activity and diminished the concentration of the endogenous antioxidant α-tocopherol (vitamin E). Moreover, amlodipine in a concentration of 2 ng/ml decreased the content of malonic dialdehyde (by-product of oxidative stress) and activity of superoxide dismutase in the blood [51].

Amlodipine antioxidant activity could be related to its as dihydropyridine compound reductant nature or hydrogen donor properties, respectively – the ability of donating protons and electrons to the lipid peroxide molecules, thereby blocking the peroxidation process. Verapamil and amlodipine produced a potent antiischemic effect and reduced the area of myocardial infarction in rats.

The observed changes were accompanied by inhibition of lipid peroxidation. In contrast to verapamil, amlodipine in a dose of 50 ng/ml *in vitro* decreased hemoglobin affinity for oxygen. Amlodipine however shows no activity on the inhibition of macrophage superoxide release and cell migration, which occurs by decreased TNFα-induced O_2- release.

g. **Amlodipine effect on some enzymes and enzyme systems in normal and tumor cells:** Amlodipine retarded THP-1 cell (monocyte/macrophage derived human leukemia cell line) adhesion to vascular endothelium via inhibition of protein kinase C signal transduction thus confirmed role of amlodipine in the mutual interaction of monocytes and endothelium, modulation proteinkinase-C and RhoA-dependent mechanisms (RhoA-small proteins of GTPase) [52].

Amlodipine and nifedipine stimulated the dead space wound healing in rats. The drugs enhanced normal healing as evidenced by increase in tensile strength of 10 days old granulation tissue. There was neither a significant change in the hydroxyproline level (or collagen) nor a change in the glycosaminoglycan content in granulation tissue. However, lysyloxidase level was increased significantly. The increase in tensile strength could thus be attributed to better cross-linking and maturation of collagen rather than collagen synthesis per se. The drugs were also able to overcome steroid depressed wound healing. It is likely that the prohealing effects may be related to the improved antioxidant status too, since superoxide dismutase (SOD) levels were observed to be higher in the CCB- treated animals [53].

Amlodipine dose-dependently (1-100 nM) inhibited p42/p44 MAPKs activation by bFGF – basal fibroblast growth factor (experiments on the human arteria smooth muscle cells), and this may be a reason for amlodipine antiproliferative activity [54, 55, 56]. In the experiments with promyelocyte leukemia HL-60 and MDR HL-60 cells amlodipine augmented stealthy liposomal topotecan (SLT) anticancer activity. The enhanced antitumor activity in the MDR HL-60 cells by the SLT plus amlodipine could be owing to multiple reasons:

a) synergistic apoptosis inducing effect,

b) reversal of MDR by amlodipine and

c) increasing the availability of active lactone of topotecan by the stealthy liposomes.

The apoptosis induced by amlodipine proceeds through initiator caspase 8 and then effectors caspases 3/7 signalling pathway [57]. The amlodipine antitumor activity *in vitro* and *in vivo* was shown in the cell culture of human epidermoid carcinoma A431 cell line, and in mice, the cell proliferation was inhibited [58]. Amlodipine caused G1 cell cycle arrest and growth inhibition in A431 cells [59].

Flow cytometric analysis revealed that treatment with amlodipine (20–30 μM, for 24 h) induced G1 phase cell accumulation. The amlodipine-induced G1 arrest was associated with a decrease in phosphorylation of pRB(retinoblastoma protein), a regulator of G1 to S phase transition, reduction of protein levels of cyclin D1 and cyclin dependent kinase 4 (CDK4), G1 specific cell cycle proteins, and increased expression of $p21^{Waf1/Cip1}$, an inhibitory protein of CDK/cyclin complexes. *In vitro* kinase assay revealed that amlodipine significantly decreased CDK2-, CDK4-, and their partners cyclin E- and cyclin D1-associated kinase activities. The amlodipine-induced reductions in cyclin D1 protein expression and in CDK2 kinase activity were reproduced by a dihydropyridine derivative, nicardipine, having an inhibitory effect on A431 cell growth, but not by nifedipine, lacking the antiproliferative activity.

The results demonstrate that amlodipine caused G1 cell cycle arrest and growth inhibition in A431 cells through induction of p21(Waf1/Cip1) expression, inhibition of CDK(cycline dependent kinase4)/cyclin-associated kinase activities, and reduced phosphorylation of pRB. Amlodipine prevented the occurence of chronic morphological vasospasm in the femoral arteria (determined by morphometric analysis in rats) [24]. Ca^{2+} channel blockers, such as amlodipine, inhibit vascular smooth muscle cell (VSMC) growth through interactions with targets other than L-type Ca^{2+} channels.

The effects of amlodipine on Ca^{2+} movements in thrombin- and thapsigargin-stimulated VSMCs were therefore investigated by determining the variations of intracellular free Ca^{2+} concentration in fura 2-loaded cultured VSMCs. Results indicated that 10–1,000 nM amlodipine inhibited thrombin-induced Ca^{2+} mobilization from a thapsigargin-sensitive pool and thapsigargin-induced Ca^{2+} responses, including Ca^{2+} mobilization from internal stores and store-operated Ca^{2+} entry.

These effects of amlodipine do not involve L-type Ca^{2+} channels and could not be reproduced with 100 nM isradipine, diltiazem, or verapamil. The inhibition by amlodipine of Ca^{2+} mobilization appears therefore to be a specific property of the drug, in addition to its Ca^{2+}channel-blocking property. It is suggested that amlodipine acts in this capacity by interacting with Ca^{2+}-ATPases of the sarcoplasmic reticulum, thus modulating the enzyme activity. This mechanism might participate in the inhibitory effect of amlodipine on VSMC growth [60]. In thrombin- and thapsigargin-stimulated SMCs, amlodipine and not isradipine dose-dependently reduced Ca^{2+} mobilization (i.e. Ca^{2+} release from internal stores); these dihydropyridines did not affect either Ca^{2+} influx or ERK 1/2 activation.

In bFGF-stimulated SMCs, amlodipine and isradipine reduced both Ca^{2+} influx and ERK 1/2 activation without affecting Ca^{2+} mobilization. ERK 1/2 activation could also be directly stimulated by the L-type channel agonist Bay K 8644, demonstrating the involvement of voltage-gated Ca^{2+} influx in this process. Most of the observed effects described were obtained with approximately 10 nmol/l amlodipine/isradipine (i.e. concentrations close to the peak plasma level in treated patients) [61].

Amlodipine is reported [58] to inhibit proliferation of human epidermoid carcinoma A431 cells. It specifically attenuated Ca^{2+}responses evoked by thapsigargin, an inhibitor of endoplasmic reticulum Ca^{2+}-ATPases. The possible mechanism of the antiproliferative action of amlodipine and its antitumor effect on A431 xenografts in nude mice was examined. Amlodipine reduced BrdU incorporation into nucleic acids in serum-starved A431 cells, and the reduction was diminished by uridine 5'-triphosphate (UTP), a phospholipase C (PLC)-linked agonist.

Fluorometric measurement of intracellular free Ca^{2+} concentration revealed that amlodipine blunted the UTP-induced Ca^{2+}release from the internal Ca^{2+} stores and consequently Ca^{2+} influx through Ca^{2+}-permeable channels on the plasma membrane. Investigations were made whether amlodipine interacts directly with intracellular Ca^{2+} stores in fluo-3-loaded cells. Application of amlodipine in Ca^{2+}-free HBSS evoked a transient rise in $[Ca^{2+}]_i$. Thapsigargin applied thereafter caused no rise in $[Ca^{2+}]_i$, and *vice versa*, suggesting that amlodipine induced Ca^{2+} release from the thapsigargin-sensitive Ca^{2+} stores.

When amlodipine was applied after UTP, an additive rise in $[Ca^{2+}]_i$ was observed, while UTP after amlodipine did not cause any increase in $[Ca^{2+}]_i$. This indicates that amlodipine caused Ca^{2+} release from both IP_3-sensitive and IP_3-insensitive Ca^{2+} stores. It were shown that thapsigargin subsequently evoked a rise in $[Ca^{2+}]_i$ by UTP, while UTP after thapsigargin did not, indicating that thapsigargin induced Ca^{2+} release from both IP_3-sensitive and IP_3-insensitive Ca^{2+} stores. In contrast, no $[Ca^{2+}]_i$ increases were induced by nicardipine or nimodipine, both of which have an antiproliferative effect on A431 cells and also blunted the thapsigargin-induced Ca^{2+} release from internal Ca^{2+} stores and the ensuing Ca^{2+} influx.

In addition, the Ca^{2+}-releasing effect of amlodipine was evoked with as low as 3 µM. Although amlodipine alone caused Ca^{2+} release from thapsigargin-sensitive Ca^{2+} stores, such an effect was not reproduced by other dihydropyridine Ca^{2+} channel blockers, including nicardipine and nimodipine, despite their antiproliferative effects in the cells. Daily intraperitoneal administration of amlodipine (10 mg/kg) for 20 days into mice bearing A431 xenografts retarded tumor growth and prolonged the survival of mice. These results suggested a potential antitumor action for amlodipine *in vitro* and *in vivo*, which may be in part mediated by inhibiting Ca^{2+} influx evoked by

the passive depletion of internal Ca^{2+} stores and by PLC-linked agonist stimulation [58]. Possibly amlodipine mechanism included the carboanhydrase I [E.C. 4.2.1.1] inhibiting activity (as the other dihydropyridines too). This enzyme catalyses reversible hydration of carbon dioxide, with the following increase of pH, which could influence entry of the calcium ions into the cell through calcium channels [62, 63, 64].

A remarkable antiulcer effect of amlodipine was observed, as well as interaction with H2 blocker and proton pump inhibitor in *pylorus* ligated rats. The similar therapeutic effect was observed by using combinations of small amlodipine and famotidine or omeprazol doses [65]. Amlodipine caused the antinociceptive action by blocking N-type Ca^{2+} channels at the primary afferent neurons and blocking synaptic transmission in the nociceptive neurons (experiments on mice) [66].

Prolonged use of the drug facilitated learning and memory in mice [67]. Amlodipine retarded the development of pulmonar hypertension (in experiments with rats), diminishing the blood pressure, blocking the decrease of the endothelial NO synthase activity, as well as the result of its own antiinflammatory, antiproliferative and antithrombotic action [68].

Amlodipine enchanced the activity of antiepileptic drugs (against pentylenetetrazole-induced seizures, in the experiments *in vivo*) [69].

h. **Inhibition of smooth muscle cell proliferation and migration:** Vascular smooth muscle cell proliferation and migration are early characteristics of atheroma development and are closely related to vascular diseases. In VSMCs obtained from atherosclerotic plaques, Ca^{2+} transport mechanisms and basal intracellular Ca^{2+} levels are disrupted as a result of increased membrane cholesterol content. These changes have important consequences for atherosclerosis, because calcium participates directly in signal transduction pathways that promote SMC proliferation and migration, among other changes. Membrane bilayer swelling was attributed to the accumulation of unesterified cholesterol in the diseased artery, an atherogenic stimulus that leads to increased SMC proliferation owing to the production of growth factors [1]. Amlodipine (in this case only its R(+) stereoisomer), has been shown to effectively inhibit smooth muscle cell proliferation following cholesterol enrichment [1]. There is growing evidence that calcium antagonists, and also amlodipine, have antiproliferative action, inhibiting VSMC growth/proliferation [34, 54].

Their molecular mechanisms were clarified. The antiproliferative action of amlodipine is achieved by induction (increasing the expression) of the $p21^{(Waf1/Cip1)}$ gene, which may explain beneficial covert effects of this widely used cardiovascular therapeutic drug beyond a more limited role as a vascular relaxant [55]. Amlodipine inhibits cell proliferation via PKD1(polycystic kidney disease 1)-related pathway [56]. Polycystic kidney disease 1 (PKD1) is also involved in cell cycle inhibition via $p21^{(Waf1/Cip1)}$ up-regulation. Authors clarified the involvement of PKD1-related signaling on human coronary artery smooth muscle cells (hCASMCs). Cultured hCASMCs, which constitutively express PKD1, were stimulated with 5% serum.

Amlodipine increased $p21^{(Waf1/Cip1)}$ expression in a dose- and time-dependent manner, resulting in reduced hCASMC proliferation. The inhibitory effect of amlodipine was mimicked by overexpression of PKD1 and was reversed by a dominant-negative version of PKD1 (R4227X). Immunoblot analysis showed that phosphorylated JAK2 was increased by amlodipine treatment or PKD1 overexpression. A luciferase assay revealed that the overexpression of PKD1 induced *STAT1* enhancer activity. These data suggest that PKD1 contributes to the antiproliferative effect of amlodipine on hCASMCs via JAK/STAT signaling and $p21^{(Waf1/Cip1)}$ up-regulation.

The study was designed [54] to determine whether mitogen-activated protein kinases (MAPKs) are involved in VSMC proliferation induced by basic fibroblast growth factor (bFGF), and to examine the inhibitory effect of amlodipine. p42/p44 MAPK is mitogen activated proteinkinase, which is related to serine/threonine protein kinases group. It phosphorylates both nuclear and cytoplasmic

proteins. MAPK calls proto-oncogene *c-fos* and *c-myc* expression in the cell nucleus, and stimulates cell growth and differentiation. Amlodipine dose-dependently (1-100 nM) inhibited p42/p44 MAPKs activation by bFGF – basal fibroblast growth factor, which have expressed mitogen properties, and which is a multifunctional growth factor.

Amlodipine (10 nM) inhibited bFGF-induced human VSMC proliferation, inhibited both short-term and sustained p42/p44 MAPKs activation by bFGF, suggesting that bFGF-induced VSMC proliferation may be related to p42/p44 MAPKs activation, and that the antiproliferative effect of amlodipine may be related to its inhibition of p42/p44 MAPKs activation. Keeping in the mind that amlodipine had antiproliferative [54] and antimicrobial [70] action, it was found that amlodipine possessed the ability to retard the development of *Leishmania donovani* (visceral leishmaniosis) infection [71], both *in vitro* (in 50% effective concentration for amlodipine and lacidipine is 2 and 2.5 µg/ml, resp.) and *in vivo* (in mice, by use of amlodipine and lacidipine commercial oral tablets from '*Sun Pharmaceuticals Ltd.'*, in the doses 10 mg/kg weight, which are 4.5-times and 325- times lower doses than $LD_{50\%}$ for amlodipine and lacidipine, respectively).

Drugs were administered once weekly during four weeks. This property is independent from influence on Ca^{2+} transport, and is determined by phenyl-1, 4-dihydropyridine cycle structure.

i. **Modulation of vascular cell gene expression and extracellular matrix formation:** Amlodipine increases the expression of the cytokine interleukin-6 in human vascular SMC by directly activating its specific gene promoter. Up-regulation of interleukin-6 by amlodipine at low pharmacological concentrations leads to potent antiproliferative effect, independent of calcium channel modulation [32]. Amlodipine was also shown to have favourable effects on the synthesis of extracellular matrix (ECM) proteins involved in atherogenesis [1, 72]. Disruptions in ECM metabolism may contribute to vascular remodelling during the development and progression of human atherosclerotic lesions.

Matrix metalloproteinases (MMPs) represent a family of enzymes that degrade ECM components in human atherosclerotic plaques, leading to promotion and destabilisation of the lesion. In both fibroblasts and vascular SMC, CCBs have been shown to inhibit the expression of procollagens I, III and IV at nanomolar concentrations [72]. Matrix metalloproteinases actively participate in VSMC proliferation and vessel-wall remodeling. Recently, various CCBs, including amlodipine, have been reported to modulate MMP activity.

Although the mechanisms of action involved are still unclear, CCBs might act not only on MMP activity/expression but also on transcription of the tissue inhibitor of metalloproteinases (TIMPs) [73]. It was hypothesised that calcium antagonists may potentiate the activity of MMPs in arterial aneurysmal disease (AAA) and thus accelerate AAA expansion. In the therapeutic ranges amlodipine significantly enhanced elastin degradation and potentiated MMP-9 activity within the aortic organ cultures [74].

j. **Influence of amlodipine on collagenolytic activity:** In the cell culture (cells of human endothelium) amlodipine, in contrary to nifedipine, significantly decreased the level and slightly inhibited expression of matrix metalloproteinase-1 (MMP-1) and its tissue inhibitor (TIMP-1) in human vascular endothelial cells (ECs), stimulated with interleukin-1 (beta) (IL-1beta), thereby significantly inhibiting collagenolytic activity. This process was not significantly influenced by nifedipine. It seems, that this action too does not depend from the blockage of the Ca^{2+} channels, because the findings revealed that amlodipine, but not nifedipine, inhibited IL-1beta-induced matrix metalloproteinase-1 expression in human ECs [73].

k. **Mitochondria as amlodipine drug target:** Ca^{2+} accumulation and Ca^{2+} overloading in mitochondria are responsible for the cell abnormality associated with ischemia and reperfusion injury. Study [75] was aimed at evaluating the efficacy of the Ca^{2+} channel blocker amlodipine on the mitochondrial Ca^{2+} accumulation, mitochondrial antioxidant status (antioxidants (glutathione) and antioxidant enzymes (superoxide dismutase, catalase)) and

mitochondrial respiratory enzymes (malate dehydrogenase, succinate dehydrogenase and NADH dehydrogenase) in ischemia and reperfusion (I/R) induced liver injury. Pretreatment with amlodipine effectively counteracted the alteration in mitochondrial enzymes induced by ischemia-reperfusion liver damage. Transmission electron microscopic (TEM) study confirmed the restoration of cellular normality and the cytoprotective role of amlodipine against I/R induced hepatic injury. On the basis of these findings it may be concluded that amlodipine not only possesses Ca^{2+} channel antagonist properties but it may also reduce the extent of mitochondrial damage by its antioxidant activity.

l. **Amlodipine effect on accumulation of advanced glycation end products AGE and RAGE**: Recent studies suggested that advanced glycation end products (AGEs) can promote the development of atherosclerotic lesions in a manner similar to oxidatively modified low density lipoproteins. Atheroprotective amlodipine effect partly was explained as the result of the inhibition of AGE accumulation [38]. Amlodipine and fluvastatin inhibited the deposition of AGE in aortic wall of rabbits fed with cholesterol (1% in the diet) and fructose (10% in water).

Although amlodipine, a calcium channel blocker, and fluvastatin, a 3-hydroxy-3-methylglutaryl CoA reductase inhibitor, show antioxidant and atheroprotective effects, the relation of AGEs to their effects is unknown. The exact mechanism of AGE suppression is ambiguous. Inhibitory effects of chronic treatment with amlodipine (5 mg/kg per day) or fluvastatin at a dose insufficient to reduce plasma cholesterol levels (2 mg/kg per day) on the accumulation of AGEs in atherosclerotic aortas of rabbits were studied immunohistochemically.

After eight weeks of treatment, AGEs, namely argpyrimidine, carboxymethyllysine and pyrraline, markedly accumulated with intimal thickening in cholesterol and fructose-fed control rabbits, while the drugs inhibited those changes other than the pyrraline deposition without plasma lipid-lowering effects. Enhanced lipid peroxidation was observed in plasma from cholesterol and fructose-fed rabbits only, and lipid peroxidation was not suppressed by the drugs.

Clinical studies: Amlodipine already has a comparatively long and succesfull history of its clinical use for the cure of hypertonia (hypertension) and coronary ischemic disease.

Analysis of several randomised studies revealed however that amlodipine changed the life span of the patients with coronary insufficience not-significantly.

For patients with vasospastic stable *angina pectoris*, amlodipine remarkably decreased the count of incidents and decreased the consumption rate of nitroglycerine, and, in comparison with nifedipine, caused less expressed negative inotropic effect [18].

For patients with arterial hypertension amlodipine improved the endothelial function and remarkably inhibited the development of *a.carotis* atherosclerosis [17].

In the wide placebo-controlled 'crossover GENRES' study was found the correlation between some fundamental laboratory analysis results and proposed amlodipine (in the dose 5 mg per day, during 4-weeks long period) hypotensive effect [76]. It was observed however remarkably individual deviations from the mean (medial) parameters. Interestingly, negative correlation between calcium level in the serum and the amlodipine effect was found [76]. About the reason of this phenomenon some speculative hypotheses were proposed. For example: calcium ions under amlodipine influence concentrate into intracellular structures – organellae.

Medial level of the electrolyte (sodium, chloride and potassium ions) excretion with urine was with the negative correlation according to the lowering of the blood pressure under the amlodipine effect. The same negative correlation was observed between main cholesterol level and the lowering of the blood pressure under the amlodipine effect. Negative correlation was observed also between marker metabolites as uric acid and insuline level and the lowering of the blood pressure under the amlodipine effect. It could be mentioned that decreased sodium intake with the food did not lower amlodipine antihypertensive effect.

Authors concluded that diuretics and CCB are more suitable for the antihypertensive therapy for the patients with hypertension characterised by low renin level.

Amlodipine was used for the long (6 months) period with success for cure of children suffering hypertonia [8, 10].

In case of patients with pulmonary hypertension amlodipine remarkably decreased the resistance of the pulmonary blood vessels, decreased pulmonary blood pressure and improved the function of the right chamber (ventricule), essentially (fundamentally) decreasing the pressure in the right auricle [77]. Organoprotective and pleiotropic effects of amlodipine (as maleate) in hypertensive patients with chronic obstructive pulmonary disease were observed [45].

After the chronical use of amlodipine the tendency could be observed to increase norepinephrine level in the plasma and to increase sympathic nerve activity, however the changes were not statistically significant, and therefore it was concluded that amlodipine practically did not change the sympathic nerve activity [78]. However in other clinical studies it was observed the increased sympathic nerve activity after medication with amlodipine (6 weak course), which accompanied the increase of the heart systolic and diastolic volume [79].

In the patients with cardiomyopathies of the non-ischaemic nature (medicated with ACE inhibitors, digoxine, diuretics) amlodipine did not influence the existing level of circulating neurohormones and marker substances of the oxidative stress [80].

In the patients with NIDDM (2nd type diabetes) amlodipine (5-10 mg, 12 weaks) not only decreased blood pressure, but improved the insuline resistance and decreased the level of TNF-alpha [81]. In the hypertensive patients with NIDDM amlodipine decreased microalbuminuria (urinary albumin excretion – UAE). In the combination with fosinopril the effect was increased as compared for the separate (solely) both drug use [82]. In the wide clinical investigations (twenty-six prospective studies were identified and 22 manuscripts were included, 802 individuals investigated) nimodipine, amlodipine and other CCBs ability to increase the fibrinolysis (by decrease of the 'euglobulin clot lysis time'– ECLT) and to increase the activity of tissue plasminogen (tPA) was shown. The elevated fibrinolytic activity is apparently caused by an increase of the tPA antigen (tPA ag) level and a decrease of the plasminogen activator inhibitor-1 antigen (PAI-1 ag). A sensitivity analysis showed that dihydropyridines, but not phenylalkylamines, exerted a fibrinolytic effect. This fibrinolytic effect is not only seen in patients with subarachnoid hemorrhage but also in hypertensive patients.

Thus, calcium antagonists increased fibrinolytic activity [19]. This may add to the beneficial pharmacological effect of calcium antagonists to prevent ischemic events in patients with hypertension and subarachnoid hemorrhage. In the nanomolar concentrations amlodipine remarkably decreased the constitutive and platelet-derived growth factor BB (PDGF-BB)-dependent incorporation of the collagen into the extracellular matrix (ECM), which is formed by vessel smooth muscles and fibroblasts. Expression of the fibrillar (I and III type) collagen and basal membrane (IV type) collagen was blocked and the proteolytic activity of the collagenase (72-kDa type IV) was increased [72].

In the multicenter, prospective, randomized, open, blinded end point CAMELOT (The Comparison of Amlodipine *vs* Enalapril to Limit Occurrences of Thrombosis) study [83], administration of amlodipine for 24 months to normotensive patients with coronary artery disease resulted in a reduction in adverse cardiovascular events, whereas no significant treatment effects were observed with enalapril.

Furthermore, the assessment of atherosclerotic plaques by intravascular ultrasonography showed no progression in plaque volume in patients receiving amlodipine [84]**.** These results indicated that not only angiotensin receptor blockers (ARBs), but also amlodipine may have antiatherosclerotic effects. In the randomized, placebo-controlled clinical investigation (coronary angiography for 825 patients) the use of amlodipine however sufficiently did not change the development of the of atherosclerosis (angiographic data), as well did not change life span or frequency of the infarct incidents, as compared with the placebo group [85].

However, clinical studies of other authors have suggested the ability (property) of amlodipine to decrease the frequency the incidents of heart and brain attack connected with ischaemia [42], and this could not be explained only by the effective antihypertensive activity [86].

Prolonged use (till one year) of amlodipine gives good results for the patients with transplanted heart, keeping the blood pressure and kidney functions in the normal level [87]. Additive action of amlodipine and atorvastatine was shown in the clinical studies with patients with hypertensia & hyperlipidemia. The synergy of both drugs beneficially influenced the cure of patients [15, 88].

The effectiveness and safety of amlodipine has been summarized in many randomized placebo-controlled clinical studies. Amlodipine is comparativelly well tolerated, if used in the doses not more than 10 mg/per day. The observed side effects are small or medium [89, 90], and the medication should be broken only for about 1.5% of patients (in the placebo group 1%).

The main and more frequent side effects are headache and peripheral edema.

The incidence or frequency (in %%) of the amlodipine side effects depended from the dose [90]:

TABLE 1. THE INCIDENCE OR FREQUENCY (IN %%) OF THE AMLODIPINE SIDE EFFECTS

Adverse Event	2.5 mg	5.0 mg	10.0 mg	Placebo
	N=275	N=296	N=268	N=520
Edema	1.8	3.0	10.8	0.6
Dizziness	1.1	3.4	3.4	1.5
Flushing	0.7	1.4	2.6	0.0
Palpitation	0.7	1.4	4.5	0.6

Other adverse experiences that were not clearly dose related but were reported with an incidence greater than 1.0% in placebo-controlled clinical trials include the following [90]:

TABLE 2. OTHER ADVERSE EXPERIENCES OF AMLODIPINE THAT WERE NOT CLEARLY DOSE RELATED

Placebo-Controlled Studies		
	Amlodipine (%) (N=1730)	Placebo (%) (N=1250)
Headache	7.3	7.8
Fatigue	4.5	2.8
Nausea	2.9	1.9
Abdominal Pain	1.6	0.3
Somnolence	1.4	0.6

More frequently the side effects are expressed for women [89, 90].
The swelling of upper extremitates for children is reported [91].

Prolonged use of amlodipine could lead to the possibility of the development of parkinsonism [92].

Sometimes (<1%) observed other side effects could be connected with various systems of the whole organism. However amlodipine could be used safely for patients with chronic diseases (in the cases of COPD, compensated not-hereditary cardiopathy, pathologies of the peripheral blood vessels, *Diabetes Mellitus* (DM), changed lipid composition) [89].

Some exclusive events with amlodipine overdosage have been reported (extremally low blood pressure, tachycardia, resp. shock and metabolic acidosis), which could be medicated with intravenous injection or infusion of calcium salts [93, 94], however it could be effective not in all cases. At intoxication by amlodipine or other CCBs it is recommended 4-aminopyridine (fampridine) use [95].

Amlodipine could be used in the combination therapy together with the other cardio- and vasoprotective acting drugs (positive data have been reported about the combinations with atorvastatin, benazepril, fosinopril, valsartan, telmisartan (TWYNSTA®), famotidine or omeprazole a.o.).

By combined amlodipine and valsartan (angiotensine-II receptor antagonist) therapy prolonged severe hypotensia could occur, which were medicated with large dosis of insuline, glucagone and naloxone [96].

Amlodipine possesses analgesic properties, and it could be used in the combination with other analgetics [97].

The internet pages http://www.drugs.com/drug-interactions/amlodipine.html(http://www.drugs.com/ drug-interactions/amlodipine.html#ixzz13YzP4sdu) and http://www.drugs.com/drug-interactions/amlodipine. html#ixzz13Z2yqsKA) indicate about 462 drugs (with 2648 brand and generic names), which interfere with amlodipine action. The major drug interaction occurs with 11 drugs, containing as active 2 substances - itraconazole, tizanidine, and assiduity and caution should be kept by use of amlodipine in combination with these drugs. This list is however not fully completed, and rise from month to month.

In this site also 4 diseases were mentioned, when amlodipine could be contraindicated - cardiogenic shock/hypotension, obstructive coronary artery disease, significantly impaired hepatic function and hepatic disease, Chf/Ami- congestive heart failure/myocard infarction.

As it was shown in the text above, amlodipine as effective therapeutics nevertheless has a number of various side effects, therefore new its analogues were and will be designed.

May be, the new original biologically active amlodipine analogues (as mentioned in [98, 99] and other) would possess some of the intrinsic for amlodipine signes of pleiotropism.

High cytotoxic activity of some of these compounds against cancerous cells (human lung fibrosarcoma HT-1080 and mouse hepatoma MG-22A), which is connected with intensive generation of the intracellular NO radicals, was revealed already.

CONCLUSION & SUMMARY: Amlodipine is the third generation calcium antagonist, however it is not only specific calcium channel blocker, but also broad spectrum or pleiotropic active drug, possessing the possibility to touch and trap some metabolic functions and systems of the various cells, tissues and organs of the whole organism. Its ability to cure the heart and vascular diseases (hypertension and atherosclerosis), whose pathogenetic factors include excessive constriction of the vessels, thrombosis, inflammation, proliferation, oxidation, confirms the drug multifunctional, or pleiotropic action.

Amlodipine influence on the membrane permeability and cholesterol deposition in the membrane, its antioxidative action, stimulation of the NO release, as well ECM regulation are not directly connected to the decrease of the resistance of the blood vessels – antihypertensive action. The cellular and molecular mechanisms of the observed amendment of the endothelium function, delay of the cardiovascular action disturbances as the result of amlodipine use are videly investigated, however up to now are not fully clear and unequivocal.

Amlodipine could be used in the combined therapy in the combination with other cardiovascular and other organs and tissues targeting drugs: there exist positive data about atorvastatin, benazepril, fosinopril, valsartan, telmisartan, famotidin or omeprazol a.o., the mutual influence is studied for about 462 drugs, and partly it is already in use.

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REFERENCES:

1. Park Chang-Gyu. Pleiotropic effect of 3rd generation L-type calcium channel antagonist. *Circulation* (Kr). Workshop, Spring 2004: 1-11. http://www.circulation.or.kr/workshop /2004spring /file/circulation/Pleiotropic%20effect%20of%203rd%20generation%20CCB.doc;http://webcache.googleusercontent.com/search?q=cache:xSc2vfQVgv4J:www.circulation.or.kr/workshop/2004spring/file/circulation/Pleiotropic%2520effect%2520of%25203rd%2520generation%2520CCB.doc+Park+Chang-Gyu+ antagonist+circulation&cd=2&hl=en&ct=clnk&lr=lang_ ru|lang_en;http://www.docstoc.com/docs/26074727 /Pleiotropic-effect-of-3rd-generation-L-type-calcium-channel-antagonist (From: hcj (Jun Wang, Prof.), Date: 2/21/2010).
2. Wandikayi C.. Matowe, Maryam Khaja, Sahar Nasser. Pleiotropic Effects of Calcium Channel Blockers: A Systematic Review. http://www.qu.edu.qa/pharmacy/research/presentation_documents/2010_QURF_WMatowe_pres.pdf
3. Omran M.O. Alhamami, Jabbar Y. AL-Mayah, Najah R. AL-Mousawi, Hayder I. J. AL- Mousawi. Pleiotropic Effects of Antihypertensive Drugs. *The Islamic University Journal* (Series of Natural Studies and Engineering) 2007; 15(1): 1-12. http//www.iugaza.edu.ps/ara/research/http://www.iugaza.edu.ps/ar/periodical/articles/natural15(1)2007p1-12.pdf

4. Kapanadze S, Dolidze N, Bakhutashvili Z, Chapidze L, Shengelia E. Pleiotropic effects of cardilopin (secondary coronary prevention). Georgian Med News. 2005; Feb; (119): 46-8.
5. Pleiotropic effects of RAAS modulation and calcium channel blockade beyond BP reduction. In: New Frontiers in CVD Risk Management: Optimizing Outcomes in Patients with Multiple Cardiovascular Risks.*vbwg.org/resources/Slide_Lecture.../48/PP/.../VBWG06-CoreMR-S01.ppt (read-only)*
6. Weintraub HS.; Basile J. The pleiotropic effects of antihypertensive agents: do they account for additional cardiovascular benefit beyond BP reduction? *Southern Medical Journal* 2008; 101(8): 818-823. http://journals.lww.com/smajournalonline/Fulltext/2008/08000/The_Pleiotropic_Effects_of_Antihypertensive.18.aspx
7. Burges RA, Dodd MG. Amlodipine. *Cardiovasc Drug Rev* 1990; 8: 25-44.
8. Neyler WG. Amlodipine. Springer, Berlin-Heidelberg-New York, 1993, 292 pp.
9. Steffen H.-M. Amlodipine - a third generation dihydropyridine calcium antagonist. *J Clin Basic Cardiol* 1999; 2: 45-52. http://www.kup.at/kup/pdf/28.pdf
10. Flynn JT. Efficacy and safety of prolonged amlodipine treatment in hypertensive children. *Pediatric Nephrol* 2005; 20(5): 631-635.
11. Fleckenstein-Grűn G, Frey M, Thimm F, Fleckenstein A. Protective effects of various calcium antagonists against experimental atherosclerosis. *J Hum Hypertens* 1992; 6 (Suppl 1): S13-S18.
12. Mason RP, Marche P, Hintze TH. Novel vascular biology of third-generation L-type calcium channel antagonists. Ancillary actions of amlodipine. *Arteriosclerosis, Thrombosis, and Vascular Biology (ATVB)* 2003; 23: 2155-2163.
13. Mason RP, Trumbore MW, Mason PE. Interactions biophysiques membranaires de l'amlodipine et proprietes antioxydantes [Membrane biophysical interaction of amlodipine and antioxidant properties]. Drugs 2000; 59: 9-16.
14. Sathish V, Ebenezar KK, Devaki T. Synergistic effect of Nicorandil and Amlodipine on tissue defense system during experimental myocardial infarction in rats. *Mol Cell Biochem* 2003; 243: 133-8.
15. Jukema JW, van der Hoorn JW. Amlodipine and atorvastatin in atherosclerosis: a review of the potential of combination therapy. *Expert Opinion on Pharmacotherapy* 2004; 5/2: 459-468.
16. Berkels R., Taubert D., Bartels H., Breitenbach Th., Klaus W., Roesen R. Amlodipine increases endothelial nitric oxide by dual mechanisms. *Pharmacol* 2004; 70: 39-45.
17. Nayler WG. Review of preclinical data of calcium channel blockers and atherosclerosis. *J Cardiovasc Pharmacol* 1999; 33: S7-S11.
18. Yan AT, Yan RT, Liu PP. Narrative review: pharmacotherapy for chronic heart failure: evidence from recent clinical trials. *Ann Intern Med* 2005; 142: 132-145.
19. Vergouwen MD, Vermeulen M, de Haan RJ, Levi M, Roos YB. Dihydropyridine calcium antagonists increase fibrinolytic activity: a systematic review. *J Cerebral Blood Flow & Metabolism* 2007; 27: 1293-1308.
20. Mayer B. Translocation of endothelial nitric oxide synthase: another feat of amlodipine, a cardiovascular jack-of-all-trades. *Cardiovasc Res* 2006; 71: 411-413.
21. http://www.drugbank.ca/drugs/DB00381
22. http://en.wikipedia.org/wiki/Amlodipine
23. a) Giuseppe Mancia, Co-Chairperson (Italy), Guy De Backer, Co-Chairperson (Belgium), Anna Dominiczak (UK), Renata Cifkova (Czech Republic), Robert Fagard (Belgium), Giuseppe Germano (Italy), Guido Grassi (Italy), Anthony M. Heagerty (UK), Sverre E. Kjeldsen (Norway), Stephane Laurent (France), Krzysztof Narkiewicz (Poland), Luis Ruilope (Spain), Andrzej Rynkiewicz (Poland), Roland E. Schmieder (Germany), Harry A.J. Struijker Boudier (Netherlands), Alberto Zanchetti (Italy). 2007 Guidelines for the management of arterial hypertension. The task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *European Heart Journal* 2007; 28: 1462–1536, doi:10.1093/eurheartj/ehm236,http://eurheartj.oxfordjournals.org/content/28/12/1462.full.pdf; *Journal of Hypertension* 2007; 25: 1751-1762;
    b) 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. *Journal of Hypertension* 2003; 21:1983-1992.
24. Iplikcioglu AC, Sav A, Erbengi T. The effect of amlodipine on chronic vasospasm in rats. *Turkish Neurosurgery* 1993; 3: 53-58.
25. Sobey CG, Dalipram R.A., Dusting GJ, Woodman OL. Impaired endothelium-dependent relaxation of dog coronary arteries after myocardial ischaemia and reperfusion: prevention by amlodipine, propranolol and allopurinol. *Br J Pharmacol* 1992; 105: 557-62.
26. Hoff PT, Tamura Y, Lucchesi BR. Cardioprotective effects of amlodipine on ischaemia and reperfusion in two experimental models. *Am J Cardiol* 1990; 66: 10H-16H.
27. Kloner RA, Hale SL, Alker KJ. Absence of haemodynamic deterioration in the presence of amlodipine following experimental myocardial infarction. *J Cardiovasc Pharmacol* 1992; 20: 837-45.
28. Asanuma H, Minamino T, Sanada S, Ogita H, Kim J, Fujita M, Hirata A, Tsukamoto O, Ogai A, Node K, Hori M, Tomoike H, Kitakaze M. A calcium channel blocker amlodipine increases coronary blood flow *via* both adenosine- and NO-dependent mechanisms in ischemic hearts. *J Mol Cell Cardiol* 2005; Oct., 39(4): 605-14.
29. Xu B., Xiao-hong L., Lin G., Queen L., Ferro A. Amlodipine, but not verapamil or nifedipine, dilates rabbit femoral artery largely through a nitric oxide- and kinin-dependent mechanism. *Br J Pharmacol* 2002; 136: 375-382.
30. Lechner W, Bergant A, Solder E, Kolle D. Effect of the calcium antagonist amlodipine on non-vascular smooth muscle exemplified by the uterus. *Wien Med Wochenschr* 1996; 146: 466-8.
31. Batova S, DeWever J, Godfraind Th, Balligand J-L, Dessy Ch, Feron O. The calcium channel blocker amlodipine promotes the unclamping of eNOS from caveolin in endothelial cells. Oxford Journals, Medicine, Cardiovascular Research 2006; 71(3): 478-485.*cardiovascres.oxfordjournals.org/content/71/3/478.full*
32. Mohler ER, Sorensen LC, Ghali JK, Schocken DD, Willis PW, Bowers JA, Cropp AB, Pressler ML. Role of Cytokines in the Mechanism of Action of Amlodipine: The PRAISE Heart Failure Trial. J Am Coll Cardiol 1997; 30(1): 35-41. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T18-3SHT4PW-1K&_user=4371290&_coverDate=07%2F31%2F1997&_rdoc=1&_fmt=high&_orig=gateway&_origin=gateway&_sort=d&_docanchor=&view=c&_searchStrId=1697859405&_rerunOrigin=google&_acct=C000062956&_version=1&_urlVersion=0&_userid=4371290&md5=257aa82820116fa50ad6aa9d0b97d48c&searchtype=a

33. Turgan N, Habif S, Kabaroglu CG, Mutaf I, Ozmen D, Bayindir O, Uysal A. Effects of the calcium channel blocker amlodipine on serum and aortic cholesterol, lipid peroxydation, antioxidant status and aortic histology in cholesterol-fed rabbits. *J Biomed Sci* 2003; Jan-Feb; 10(1): 65-72.
34. Umemoto S, Kawahara S, Hashimoto R, Umeji K, Umemoto S, Matsuda S, Kawahara S, Tanaka M. Different effects of amlodipine and enalapril on the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway for induction of vascular smooth muscle cell differentiation *in vivo*. *Hypertens Res* 2006; 29: 179- 86.
35. Yoshii T, Iwai M, Li Z, Chen R, Ide A, Fukunaga S, Oshita A, Mogi M, Higaki J, Horiuchi M. Regression of atherosclerosis by amlodipine via anti-inflammatory and anti-oxidative stress actions. *Hypertens Res* 2006; 29(6): 457-466.
36. Trion A, Maat M, Jukema W, Maas A, Offerman E, Havekes L, Szalai A, van der Laarse A, Princen H, Emeis J. Anti-atherosclerotic effect of amlodipine, alone and in combination with atorvastatin, in APOE*3-Leiden/hCRP transgenic mice. *J Cardiovasc Pharmacol* 2006; 47(1): 89-95.
37. van de Poll SW, Delsing DJ, Jukema JW, Princen HM, Havekes LM, Puppels GJ, van der Laarse A. Raman spectroscopic investigation of atorvastatin, amlodipine, and both on atherosclerotic plaque development in APOE*3 Leiden transgenic mice. *Atherosclerosis* 2002; 164(1): 65-71.
38. Akira K, Amano M, Okajima F, Hashimoto T, Oikawa Sh. Inhibitory effects of amlodipine and fluvastatin on the deposition of advanced glycation end products in aortic wall of cholesterol and fructose-fed rabbits. *Biol Pharm Bull* 2006; 29(1): 75.
39. Mohammadi M, Mirzaei F, Badalzadeh R. Effect of amlodipine on blood and aortic tissue concentration of endothelin in male rabbits receiving atherogenic diet. *Indian J Pharmacol* 2007; 39(6): 276-280.
40. Survase S, Ivey M E, Nigro J, Osman N, Little P J. Actions of calcium channel blockers on vascular proteoglycan synthesis: relationship to atherosclerosis. *Vasc Health Risk Manag* 2005; September; 1(3):199–208.http://www.ncbi.nlm.nih. gov/pmc/ articles/PMC1993947/
41. Wang WZ, Matsumori A, Yamada T, Shioi T, Okada I, Matsui S, Sato Y, Suzuki H, Shiota K, Sasayama S. Beneficial effects of amlodipine in a murine model of congestive heart failure induced by viral myocarditis. A possible mechanism through inhibition of nitric oxide production. *Circulation* 1997; 95: 245-51.
42. Craig W. Amlodipine prevents angina, not atherosclerosis. *Pharmacother* 2002; 22(3): 400-402.
43. Candido R, Allen TJ, Lassila M, et al. Irbesartan but not amlodipine suppresses diabetes-associated atherosclerosis. *Circulation* 2004; 109: 1536-1542.
44. Guorong MA, Allan TJ, Cooper ME, Zemin Cao. Calcium channel blockers, either amlodipine or mibefradil, ameliorate renal injury in experimental diabetes. *Kidney Int* 2004; 66(3): 1090-8.
45. Adasheva T. V., Zadionchenko V. S., Pavlov S. V., Fedorova I. V., Matsievich M. V., Poryvkina O. N., Li V. V., Mironova M. A. Organoprotective and *pleiotropic* effects of *amlodipine* in hypertensive patients with chronic obstructive pulmonary disease. Журнал *«Сердце»* (Journal *„Heart"*), 2010г., №3 - ОССН,http://www.ossn.ru,*medic.ossn.ru/publications /magazine/1448/* - (in Russian).
46. Shankar RP, Bhargava VK, Grover A, Mazumdar S, Garg SK. Amlodipine, Nitric oxide and platelet aggregation. *Asian Cardiovasc Thorac Ann* 2000; 8: 357-360.
47. Batova S, DeWever J, Godfraind Th, Balligand J-L, Dessy C, Feron O. The calcium channel blocker amlodipine promotes the unclamping of eNOS from caveolin in endothelial cells. *Cardiovasc Res* 2006; 71(3): 478-85.
48. Xiaoping Zhang, Hintze TH. Amlodipine releases nitric oxide from canine coronary microvessels: an unexpected mechanism of action of a calcium channel-blocking agent. *Circulation* 1998; 97: 576-580.
49. Zhang X., Recchia FA, Bernstein R, Xu X., Nasjletti A, Hintze TH. Kinin-mediated coronary nitric oxide production contributes to the therapeutic action of angiotensin-converting enzyme and neutral endopeptidase inhibitors and amlodipine in the treatment in heart failure. *Pharmacol* 1999; 288(2): 742-751.
50. Franzoni F, Santoro G, Regoli F, Plantinga Y, Femia FR, Carpi A, Galetta F. An *in vitro* study of the peroxyl and hydroxyl radical scavenging capacity of the calcium antagonist amlodipine. *Biomed Pharmacother* 2004; 58: 423-426.
51. Gatsura SV. Oxygen-dependent mechanisms underlying the antiischemic effect of verapamil and amlodipine. *Bull Exp Biol Med* 2004; 137: 40-42.
52. Yu T, Morita I, Shimokado K, Iwai T, Yoshida M. Amlodipine modulates THP-1 cell adhesion to vascular endothelium via inhibition of protein kinase C signal transduction. *Hypertension* 2003; 42: 329-334.
53. Bhaskar HN, Udupa SL, Udupa AL. Effect of nifedipine and amlodipine on dead space wound healing in rats. *Indian J Exp Biol* 2005; 43: 294-6.
54. Zhang Y-Z, Gao P-J, Wang X-Y, Stepien O, Marche P, Zhang Z-L, Zhu D-L. The ihibitory mechanism of amlodipine in human vascular smooth muscle cell proliferation. *Hypertens Res* 2000; 23: 403-406.
55. Ziesche R, Petkov V, Lambers C, Erne P, Block LH. The calcium channel blocker amlodipine exerts its anti-proliferative action *via* p21(Waf1/Cip1) gene activation. *FASEB J* 2004; 18: 1516-23.
56. Ohba T, Watanabe H, Murakami M, Radovanovic M, Iino K, Ishida M, Tosa Sh, Ono K, Ito H. Amlodipine inhibits cell proliferation via PKD1-related pathway. *Biochem Biophys Res Commun* 2008; 369(2): 376-381.
57. Li X, Lu L, Liang GW, Ruan GR, Hong HY, Long C, Zhang YT, Liu Y, Wang JC, Zhang X, Zhang Q. Effect of stealthy liposomal topotecan plus amlodipine on the multidrug-resistant leukemia cells/in vitro/ and xenograft in mice. *Eur J Clin Investig* 2006; 36: 409.
58. Yoshida J, Ishibashi T, Nishio M. Antitumor effects of amlodipine, a Ca^{2+} channel blocker, on human epidermoid carcinoma A431 cells in vitro and in vivo. *Eur J Pharmacol* 2004; 492(2-3): 103-112.
59. Yoshida J, Ishibashi T, Nishio M. G1 cell cycle arrest by amlodipine, a dihydropyridine Ca^{2+} channel blocker, in human epidermoid carcinoma A431 cells. *Biochem Pharmacol* 2006; 73(7): 943-953.
60. Stepien O, Marche P. Amlodipine inhibits thapsigargin-sensitive Ca^{2+} stores in thrombin-stimulated vascular smooth muscle cells. *Am J Physiol Heart Circ Physiol* 2000; 279: H1220-H1227.
61. Stepien O, Zhang Y, Zhu D, Marche P. Dual mechanism of action of amlodipine in human vascular smooth muscle cells. *J Hypertens* 2002; 20: 95-102.
62. Puscas I, Gilau L, Coltau M, Pasca R, Domuta G, Baican M, Hecht A: Hypotensive effect of calcium channel blockers is parallel with carbonic anhydrase I inhibition. *Clin Pharmacol Ther* 2000 Oct; 68(4): 443-9.
63. Puscas L, Gilau L, Coltau M, Pasca R, Domuta G, Baican M, Hecht A: Calcium channel blockers reduce blood pressure in

part by inhibiting vascular smooth muscle carbonic anhydrase I. *Cardiovasc Drugs Ther* 2000 Oct; 14(5): 523-8.
64. Puscas I, Coltau M, Baican M, Pasca R, Domuta G, Hecht A: Vasoconstrictive drugs increase carbonic anhydrase I in vascular smooth muscle while vasodilating drugs reduce the activity of this isozyme by a direct mechanism of action. *Drugs Exp Clin Res* 2001; 27(2): 53-60.]
65. Bhave AL, Bhatt JD, Hemavathi KG. Antiulcer effect of amlodipine and its interaction with H2 blocker and proton pump inhibitor in pylorus ligated rats. *Indian J Pharmacol* 2006; 38:403-407.
66. Murakami M, Nakagawasai O, Fujii Sh, Kameyama K, Murakami Sh, Hozumi S, Esashi A, Taniguchi R, Yanagisawa T, Tan-no K, Tadano T, Kitamura K, Kisara K. Antinociceptive action of amlodipine blocking N-type Ca^{2+} channels at the primary afferent neurons in mice. *Eur J Pharmacol* 2001; 419(2-3): 175-181.
67. Quartermain D. Chronic administration of the Ca^{2+} channel blocker amlodipine facilitates learning and memory in mice. *Eur J Pharmacol* 2000; 399(1): 57-63.
68. Mawatari E, Hongo M, Sakai A, Terasawa F, Takahashi M, Yazaki Y, Kinoshita O, Ikeda U. Amlodipine prevents monocrotaline-induced pulmonary arterial hypertension and prolongs survival in rats independent of blood pressure lowering. *Clin & Exper Pharmacol Physiol* 2007; 34(7): 594-600.
69. Kaminski RM, Mazurek M, Turski WA, Kleinrok Z, Czuczwar SJ. Amlodipine enchances the activity of antiepileptic drugs against pentylenetetrazole-induced seizures. *Pharmacol Biochem Behav* 2001; 68: 661-8.
70. Kumar KA, Ganguly K, Mazumdar K, Dutta NK, Dastidar SG, Chakraborty AN. Amlodipine: a cardiovascular drug with powerful antimicrobial property. *Acta Microbiol Pol* 2003; 52: 285-292.
71. Palit P, Ali N. Oral therapy with amlodipine and lacidipine, 1,4-dihydropyridine derivatives showing activity against experimental visceral leishmaniasis. *Antimicrobial Agents Chemother* 2008; 52(1): 374-377.
72. Roth M, Eickelberg O, Kohler E, Erne P, Block LH. Ca^{2+} channel blockers modulate metabolism of collagens within the extracellular matrix. *Proc Natl Acad Sci USA* 1996; 93(11): 5478-5482.
73. Ikeda U, Hojo Y, Ueno S, Arakawa H, Shimada K. Amlodipine inhibits expression of matrix metalloproteinase-1 and its inhibitor in human vascular endothelial cells. *J Cardiovasc Pharmacol* 2000; 35(6): 887-890.
74. Boyle JR, Loftus IM, Goodall S, Crowther M, Bell PR, Thompson MM. Amlodipine potentiates metalloproteinase activity and accelerates elastin degradation. *Eur J Vasc Endovasc Surg* 1998, 16: 408-14.
75. Pronobesh C, Dagagi AV, Pallab C, Kumar WA. Protective role of the calcium channel blocker amlodipine against mitochondrial injury in ischemia and reperfusion injury of rat liver. *Acta Pharm* 2008, 58: 421-428.
76. Suonsyrjä T, Hannila-Handelberg T, Paavonen KJ, Miettinen HE, Donner K, Strandberg T, Tikkanen I, Tilvis R, Pentikäinen PJ, Kontula K, Hiltunen TP. Laboratory tests as predictors of the antihypertensive effects of amlodipine, bisoprolol, hydrochlorothiazide and losartan in men: results from the randomized, double-blind, crossover GENRES Study. *J Hypertens* 2008; 26(6): 1250-1256. http://pt.wkhealth.com /pt/re/merck/fulltext.00004872-200806000-00027.htm; jsessionid=M2XYQBJKVJ936hg2nthRrGL1wJN2HQG5L44jbGxxlNwp0V8jphSK!-929085697!181195628!8091!-1?nav=reference
77. Franz I-W, Van Der Meyden J, Schaupp S, Tonnesmann U. The effect of amlodipine on excersise-induced pulmonary hypertension and right heart function in patients with chronic obstructive pulmonary disease. *Zeitschrift fűr Kardiologie (Z Kardiol)* 2002; 91(10): 833-839.
78. Binggeli C, Corti R, Sudano I, Luscher ThF, Noll G. Effects of chronic calcium channel blockade on sympathetic nerve activity in hypertension. *Hypertension* 2002; 39: 892-903.
79. Lindqvist M, Kahan T, Melcher A, Ekholm M, Hjemdahl P. Long-term calcium antagonist treatment of human hypertension with mibefradil or amlodipine increases sympathetic nerve activity. *J Hypertens* 2007; 25(1): 169-175.
80. Wijeysundera HC, Hansen MS, Stamon E, Cropp AS, Hall C, Dhalla NS, Ghali J, Rouleau JL. Neurohormones and oxidative stress in nonischemic cardiomyopathy: relationship to survival and the effect of treatment with amlodipine. *Am Heart J* 2003; 146: 291-7.
81. Ersoy C, Imamoğlu S, Budak F, Tuncel E, Ertürk E, Oral B. Effect of amlodipine on insulin resistance & tumor necrosis factor-alpha levels in hypertensive obese type 2 diabetic patients. *Indian J Med Res* 2004; 120: 481-488.
82. Fogari R, Preti P, Zoppi A., Rinaldi A, Corradi L, Pasotti C, Poletti L, Marasi GL, Derosa G, Mugellini M, Voglini C, Lazzari P, Effects of amlodipine fosinopril combination on microalbuminuria in hypertensive type 2 diabetic patients. *Am J Hypertens* 2002; 15(12): 1042-1049.
83. Nissen SE, Tuzcu EM, Libby P, et al., for the CAMELOT Investigators. Effect of antihypertensive agents on cardiovascular events in patients with coronary disease and normal blood pressure: The CAMELOT study: A randomized controlled trial. *JAMA* 2004; 292: 2217-2225.
84. Brener SJ, Ivanc TB, Polisczuk R, et al. Antihypertensive therapy and regression of coronary artery disease: Insights from the Comparison of Amlodipine versus Enalapril to Limit Occurrences of Thrombosis (CAMELOT) and Norvasc for Regression of Manifest Atherosclerotic Lesions by Intravascular Sonographic Evaluation (NORMALISE) trials. *Am Heart J.* 2006; 152: 1059-1063.
85. Pitt B, Byington RP, Furberg CD, Hunninghake DB, Mancini GBJ, Miller ME, Riley W. Effect of amlodipine on the progression of atherosclerosis and the occurence of clinical events. *Circulation* 2000; 102: 1503-1510.
86. Angeli F, Verdecchia P, Reboldi GP, Gattobigio R, Bentivoglio M, Staessen JA, Porcellati C. Calcium channel blockade to prevent stroke in hypertension. *Am J Hypertens* 2004; 17: 817-822.
87. Leenen FH, Coletta E, Davies RA. Prevention of renal dysfunction and hypertension by amlodipine after heart transplant. *Am J Cardiol* 2007; 100(3): 531-535.
88. Leibovitz E, Beniashvili M, Zimlichman R, Freiman A, Shargorodsy M, Gavish D. Treatment with amlodipine and atorvastatin have additive effect in improvement of arterial compliance in hypertensive hyperlipidemic patients. *Am J Hypertens* 2003; 16: 7715-718.
89. Monson K, Schoenstadt A. Amlodipine side effects. *MedTV Health Information*. Last update June 03, 2008.
90. http://www.drugs.com/sfx/amlodipine-side-effects.html
91. Wong W. Amlodipine-induced bilateral upper extremity edema. *Ann Pharmacother* 2007; 41(9): 1536-1538.
92. Sempere AP, Duarte J, Cabezas C, Coria F, Claveria LE. Parcinsonism induced by amlodipine. *Movement Disorders* 2004; 10(1): 115-116.
93. Poggenborg RP, Videbaek L, Jacobsen IA. A case of amlodipine overdose. Basic *Clin Pharmacol Toxicol* 2006; 99(3): 209-212.

94. Hung YM, Olson KR. Acute amlodipine overdose treated by high dose intravenous calcium in a patient with severe renal insufficience. *Clin Toxicol (Phila)* 2007; 45(3): 301-3.
95. Wilffert B, Boskma RJ, van der Voort PH, Uges DR, van Roon EN, Brouwers JR. 4-Aminopyridine (fampridine) effectively treats amlodipine poisoning: a case report. J *Clin Pharm Ther* 2007; 32(6): 655-7.
96. Smith SW, Ferguson KL, Hoffman RS, Nelson LS, Greller HA. Prolonged severe hypotension following combined amlodipine and valsartan ingestion. *Clin Toxicol* 2008; 46(5): 470-474.
97. Dogrul A, Deniz G. The analgesic effect of amlodipine and its interaction with morphine, ketorolac and naloxone after peripheral and central administration in mice. *Eur Neuropsychopharmacol* 1996; 6(suppl.1): S1/30-S1/30(1).
98. Sīle (Beķere) L. Synthesis and enantioseparation of biologically active amlodipine analogues. Summary of the promotional work (Dr.chem.). Riga, Latvian University, Faculty of the Chemistry, 2008, 20 pp. [In Latvian]. (Bioloģiski aktīvu amlodipīna analogu sintēze un enantiosadalīšana. Promocijas darba kopsavilkums. Rīga, Latvijas Universitāte, Ķīmijas fakultāte, 2008, 20 lpp.)
99. Bekere L., Krauze A., Dambrova M., Vilskersts R., Shestakova I., Domracheva I., Kalme Z., Duburs G. Synthesis and L-type calcium channel blocking activity of 2-aminoethoxymethyl-4-aryl-1,4-dihydropyridine-3,5-dicarboxylates. In: Balticum Organicum Syntheticum, International Conference on Organic Synthesis, June 27 – 30, 2010, Riga, Latvia. Abstract PO10,56.

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