(x ± SEM; n = 15 experiments). This was significantly ($p < 0.05$) different from the control value, which averaged 2.8 ± 0.3 days (n = 15). Additional analysis of human atherectomy specimens, from 32 coronary and 32 femoral plaques of primary (45) and restenotic (19) origin, showed SMC outgrowth in 43/64 cases (67%). Interestingly, previous AI therapy was followed by significantly ($p < 0.05$) lower success, whereas medication with β-blockers, Ca-antagonists, nitrates, aspirin or antilipids had no effect. Complementary experiments using isolated human and rat SMCs revealed no inhibition for benazeprilat (24 h preincubation) on growth curves and chemokinesis of these cells.

We conclude that long-term ACE inhibition strongly induces apoptosis of vascular SMCs which apparently explains the modified SMC outgrowth activity. Our data suggest ACE inhibitors to be attractive candidates for the regulation of growth processes *via* apoptosis, though previous clinical trials (without pretreatment period) have as yet failed to prevent restenosis.

## 1043-100 Ion Channel Modulators Differentially Regulate Proliferation and Apoptosis of Human Plaque Smooth Muscle Cells

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Proliferation of smooth muscle cells (SMCs) is considered to be a central mechanism involved in human arteriosclerosis, which is possibly modulated by transmembrane ion fluxes. Therefore, using SMCs cultivated from human plaque tissue removed by directional atherectomy, we screened different ion channel modulators for their potential, antagonistic effects on SMC proliferation. Growth curves were based upon directly measured cell signals (CASY 1), to quantitate population doubling rates (PDRs). For the testing of ion channel modulators, the PDRs of treated SMCs were compared to those of control cells (= 100%):

| Ion Channel Modulator | Agent | Conc. | PDR |
|---|---|---|---|
| $Ca^{2+}$ channel agonist | Bay K 8644 | $10^{-8}$ M | 174%* |
| | | $10^{-7}$ M | 205%** |
| $Ca^{2+}$ channel antagonist | nicardipine | $10^{-8}$ M | 85% ns |
| | | $10^{-7}$ M | 78%* |
| | | $10^{-5}$ M | 47%** |
| $K^+$ channel activator | nicorandil | $10^{-8}$ M | 77%* |
| | | $10^{-7}$ M | 49%** |

(ns = not significant; *$p < 0.05$; **$p < 0.01$)

Our experimental data show that, even in low concentrations, these agents directly and differentially modulate the replicative capacity of human plaque SMCs. Additional transmission electron microscopic analysis revealed extensive cell shrinkage, membrane blebbing and formation of apoptotic bodies in SMCs treated with effective, inhibitory doses of nicardipine or nicorandil, signalling typical features of apoptosis.

In conclusion, our experimental results show the inhibitory effects of ion channel modulators on SMC proliferation *in vitro* to be attributable mainly to apoptosis. The described model may be helpful in designing and monitoring candidates for focal drug delivery, before more complex, animal models are used.

## 1043-101 Amiodarone Inhibits DNA and Protein Synthesis in Human Cardiac Fibroblasts

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Little is known about the effect of Amiodarone, a class III anti-arrhythmic drug on the extracellular matrix in the heart. Amiodarone antagonizes thyroid hormones in various tissues including the heart. We previously showed that the proliferative capacity of cardiac fibroblasts, the matrix-producing cells is regulated by thyroid hormones. In this study we compared the effect of Amiodarone with that of thyroid hormones on DNA and protein synthesis in human cardiac fibroblasts. Cultured human cardiac fibroblasts were prepared from left ventricular tissue of extirpated heart. Cells were treated (24 h) with Amiodarone (5 μg/ml), 3,3',5-triiodothyronine ($T_3$) (10 nM) or equivalent volume of distilled water (control). The synthesis of DNA and protein was determined by measuring the incorporation of $^3$H-thymidine and $^3$H-leucine in cardiac fibroblasts, respectively. The results showed a decrease in the rate of DNA (64%, $p < 0.05$) and protein synthesis (36%, $p < 0.05$) in Amiodarone-treated cells. $T_3$-treatment, however, led to 40% ($p < 0.05$) increase in DNA synthesis and 30% ($p < 0.05$) decrease in protein synthesis. Amiodarone-treatment of cells in conjunction with $T_3$ led to a net increase in DNA synthesis and net value of protein synthesis comparable to that in control cells. These data indicate that Amiodarone is an inhibitor of proliferative capacity and metabolic activity of cardiac fibroblasts, thereby a regulator of matrix production. They also point to a functional antagonism between Amiodarone and thyroid hormones. The reversal of amiodarone-induced inhibition of DNA synthesis by thyroid hormones may have clinical relevance in cardiac patients with thyroid gland dysfunction.

## 1043-102 Antioxidant Dietary Supplementation With Vitamin E Decreased Atherosclerosis and Cellular Proliferation in Lipid-Fed Rabbits Exposed to Passive Smoking

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To evaluate the effects of antioxidants on atherogenesis in a rabbit model of hypercholesterolemia and passive smoking, 32 New Zealand white male rabbits were randomly divided into 4 groups (8 each): Smoke with vitamin E (S + E) or without E (S); No-smoke with vit E (C + E) or without E (C). All four groups were fed 0.3% cholesterol diet for 13 wks. Two vitamin groups were given dietary vit E 1000 IU and beta-carotene 600 mg/kg chow for 21 wks (pretreated 8 wks before receiving lipid diet). Two groups were exposed to passive smoking (4 cigarettes/15 min, 6 hours/day) for 10 wks. Alpha-Tocopherol concentrations were measured in serum, aorta and omentum. Histomorphometry and immunohistochemistry were performed on segments of thoracic aorta using computerized planimetry and specific antibodies.

| Group | Alpha-Tocopherol | | | Aorta | | Actin |
|---|---|---|---|---|---|---|
| | Serum (mg/dl)* | Aorta (μg/g)** | Omentum (μg/g)** | Intima ($mm^2$)* | I/M (%)* | (grade)# |
| S | 2.8 ± 0.6 | 19 ± 2 | 18 ± 2 | 0.14 ± 0.05 | 4.2 ± 1.6 | 2.5 ± 0.7 |
| S + E | 4.2 ± 1.0 | 52 ± 9 | 108 ± 9 | 0.04 ± 0.03 | 1.5 ± 1.3 | 0.3 ± 0.2 |
| C | 1.4 ± 0.2 | 10 ± 1 | 13 ± 3 | 0.32 ± 0.19 | 8.9 ± 5.4 | 1.9 ± 0.7 |
| C + E | 3.6 ± 0.9 | 54 ± 13 | 121 ± 11 | 0.03 ± 0.12 | 1.2 ± 0.5 | 2.1 ± 0.7 |

Values are Mean ± SEM, *$p < 0.06$, *$p < 0.05$, **$p < 0.001$ for E; #$p = 0.05$ for E × S interaction)

Antioxidant dietary supplementation with vitamin E significantly increased alpha-tocopherol concentrations in omentum, aorta and serum; decreased intima lesions (fatty streaks), intima/media (I/M) ratio, and attenuated migration of smooth muscle cells (alpha-actin) which was enhanced by passive smoking.

Conclusion: Antioxidant dietary supplementation with vitamin E decreases atherosclerosis and cellular proliferation in the aorta of hypercholesterolemic rabbits exposed to passive smoking.

## 1043-103 Effect of Non-Selective Protein Tyrosine Kinase Blocker on Porcine Aortic, Human Arterial, and Atheroma-Derived SMC's Proliferation

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Accelerated proliferative response of smooth muscle cells (SMC's) to vessel wall injury is the principal cause of premature coronary occlusion in patients undergoing heart transplantation, coronary artery bypass grafting, and PTCA. Protein tyrosine kinases (PTK) activity is involved in multiple steps of signal transduction of SMC's growth factors. It is essential for normal cell proliferation, and greatly amplified in proliferative disorders. Blocking the activity of tyrosine kinases may provide a unique and useful strategy for the treatment of syndromes involving accelerated proliferation of vascular SMC's. We examined the inhibitory effect of AG-17, a potent non-specific PTK blocker, on porcine aortic SMC's, human internal mammary artery (IMA) SMC's and human SMC's derived from carotid artery atheromas. 1 uM, 10 uM or 100 uM AG-17 dissolved in 0.1% DMSO was added to the cultures 1, 3 and 5 days after seeding. On day 7 cultures were washed and cells were allowed to recover. Control cultures were treated with 0.1% DMSO. Cells were counted on days 7 and 15. The degree of inhibition of SMC's proliferation is shown in the table. As shown, AG-17 is a very effective inhibitor of SMC's growth. Porcine SMC's were more sensitive to the inhibitory effect of AG-17. 10 uM caused 92% inhibition and 100 uM was toxic to the cells. The inhibition of porcine SMC's proliferation was not reversible, and the cells did not resumed proliferation after day 7. 100 uM AG-17 caused 92% and 70% inhibition of human IMA and human atheroma SMC's respectively and had no toxic effect on the cells. This inhibition of human SMC's was reversible.

% Inhibition of SMC's Proliferation:

| SMC's | 1 μM | 10 μM | 100 μM |
|---|---|---|---|
| Porcine | 45% | 92% | 100% |
| IMA | 3% | 60% | 92% |
| Atheroma | 5% | 34% | 70% |