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**EFFECT OF BESTATIN ON ANGIOTENSIN I-III- AND III-INDUCED STIMULATION OF COLLAGEN GEL CONTRACTION IN CARDIAC FIBROBLASTS**  

The purpose of this investigation was to determine whether the aminopeptidase inhibitor bestatin and the angiotensin converting enzyme inhibitor lisinopril affect the angiotensin I (ANGI),angiotensin II (ANGII) or angiotensin III (ANGIII) stimulated collagen gel contraction in cardiac fibroblasts. Cardiac fibroblasts (from normal male adult rats) from passage 2 were cultured to confluency and added to a hydrated collagen gel in a Dulbecco’s Modified Eagle’s Medium without fetal bovine serum and incubated for 3 days at 37°C: ANGI (1microM), ANGII (0.1microM) and ANGIII (0.1microM) stimulated collagen gel contraction in cardiac fibroblasts after 3 days of incubation in serum-free conditions, respectively by 30.4 ± 4.8 (SEM) %, 27.1 ± 3.1 % and 15.4 ± 3.6% (n=5). The ANGI- and ANGII-induced stimulation of collagen gel contraction was of the same order but more pronounced (p<0.05) than the ANGII-stimulated collagen gel contraction. When a neutralizing antibody to TGF-bet1a was added to the collagen gel simultaneously with ANGII or ANGII or after preincubation for 1 hour before addition of ANGII or ANGII, the ANGII- or ANGII-stimulated contraction of collagen gel by cardiac fibroblasts was not affected. Addition of bestatin (0.1mM), lisinopril (10microM) or bestatin plus lisinopril did not affect the basal collagen gel contraction in cardiac fibroblasts. The ANGI-, ANGII- and ANGIII-stimulated collagen contraction was however reduced by bestatin (p<0.05) and by bestatin plus lisinopril (p<0.05) but not by lisinopril alone. Beta-aminopropionitrile (3mM), an inhibitor of lysyl oxidase, completely abolished the basal as well as the ANGI-, ANGII- and ANGIII-stimulated collagen contraction in cardiac fibroblasts. Our data suggest that aminopeptidases are involved in the ANGII-, ANGII- and ANGIII-induced stimulation of collagen contraction in cardiac fibroblasts.

**Key Words:** Collagen, Cardiac fibroblasts, Contraction

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**EFFECT OF TRANSFORMING GROWTH FACTOR-BETA1 ON CONTRACTILE ACTIVITY OF CARDIAC FIBROBLASTS IN A THREE-DIMENSIONAL COLLAGEN LATTICE**  

Myofibroblasts and transforming growth factor-beta1 (TGF-beta1) are key elements of cardiac tissue fibrosis development. The aim of this study was to determine whether the ability of TGF-beta1 to affect the contractile activity of cardiac fibroblasts depends on their differentiation into myofibroblasts. Cardiac fibroblasts (from male adult Wistar rats) from passage 2 were therefore cultured to confluency and incubated on a hydrated collagen gel with and without TGF-beta1 (0,20,40,100,200,400 or 600 pmol/L) for 1, 2 and 3 days in a Dulbecco’s Modified Eagle’s Medium (DMEM) without fetal bovine serum (FBS). Growing cultures of cardiac fibroblasts were obtained by incubating second passage fibroblasts in DMEM with 10% FBS with or without TGF-beta1 ( 0 to 600 pmol/L) for 6 days. These fibroblasts were then further incubated on the collagen gel for 1, 2 and 3 days in DMEM without FBS. TGF-beta1 increased dose-dependently the contraction of collagen gel mediated by cardiac fibroblasts, either added directly to the gel or after growing of the cardiac fibroblasts in the presence of TGF-beta1 for 6 days; reaching a maximal effect at 100 pmol/L TGF-beta1. In both culturing conditions TGF-beta1 also stimulated the tritium-thymidine incorporation and the total protein content in the cardiac fibroblasts in the collagen gel lattice. TGF-beta1 dose-dependently induced an increase in alpha-smooth muscle actin, a marker of myofibroblasts, in both culturing conditions. The TGF-beta1 induced reduction of area of the collagen gel was negatively correlated to the TGF-beta1 evoked appearance of alpha-smooth muscle actin in the collagen gel matrix. TGF-beta1 increased the contractile activity of adult rat cardiac fibroblasts and their ability to differentiate into myofibroblasts. Because contractile activity was correlated with differentiation, the influence of TGF-beta1 on cardiac fibroblast-induced collagen gel contraction may depend on the promotion of myofibroblast differentiation.

**Key Words:** collagen contraction, cardiac fibroblasts, transforming growth factor-beta1

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**EFFECT OF PROTEIN KINASE C ISOFORMS ON COLLAGEN GEL CONTRACTION AND COLLAGEN PRODUCTION IN CARDIAC FIBROBLASTS**  

The aim of this study was to investigate whether protein kinase C (PKC) inhibitors such as staurosporine, calphostin C, deaqualinium, rottlerin, hispidin, the PKC translocation inhibitor peptide or myristoylated PKC-α, γ, θ pseudo-substrates affect the collagen gel contraction or the collagen production in cardiac fibroblasts. Cardiac fibroblasts (from normal male adult rats) from passage 2 were cultured to confluency and added to a hydrated collagen gel and incubated for 3 days in serum-free conditions. Soluble collagen has been measured in the conditioned medium and nonsoluble collagen in the cell layer by assaying 3H-Proline incorporation. As compared to control, the collagen gel contraction in cardiac fibroblasts (n = 4) was dose-dependently inhibited by staurosporine, a broad spectrum PKC inhibitor; by calphostin C, an inhibitor of PKC-α,θ and γ, by deaqualinium a PKCθ inhibitor and by rottlerin, a PKCθ inhibitor. However the PKCθ inhibitor hispidin and the PKC-θ pseudosubstrates did not affect the collagen gel contraction in cardiac fibroblasts. At 10⁻⁶ M staurosporine, calphostin C, deaqualinium and rottlerin the % inhibition averaged, respectively, 92.5 ± 2.2 (SEM) %, 97.2 ± 1.1 %, 28.8 ± 0.6 % and 73.2 ± 0.6 %.

Staurosporine, calphostin C, deaqualinium and rottlerin (10⁻⁶ M) also inhibited soluble collagen production by 64.7 ± 2.9 %, 76.9 ± 3 %, 45.3 ± 2.6 % and 65.7 ± 2.2 %, respectively, and the nonsoluble collagen production by 79.3 ± 5.0 %, 95.3 ± 2.1 %, 60.4 ± 0.6 % and 70.2 ± 8.2 %, respectively, in cardiac fibroblasts in culture. Our data suggest that especially the PKC isozymes α, γ and θ are involved in the collagen gel contraction and collagen production in cardiac fibroblasts while PKCβ,γ,θ and ξ are not.

**Key Words:** Collagen, Fibroblast, Cardiac

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**MILDLY OXIDIZED LOW-DENSITY LIPOPROTEIN ACTS SYNERGISTICALLY WITH ANGIOTENSIN II IN INDUCING VASCULAR SMOOTH MUSCLE CELL PROLIFERATION**  
Takuya Watanabe, Takashi Katagiri, Rajabah Pakala, Claude R. Benedict. Third Department of Internal Medicine, Showa University School of Medicine, Tokyo, Japan; Department of Internal Medicine, Division of Cardiology, University of Texas-Houston Health Science Center, Houston, TX.

Considerable attention has been focused on both mildly oxidized low-density lipoprotein (mox-LDL) and highly oxidized LDL (ox-LDL) as important risk factors for cardiovascular disease. Further, angiotensin II (Ang II) appears to play a crucial role in the development of hypertension and atherosclerosis. We assessed the effect of oxidatively modified LDL and its major oxidative components, i.e., hydrogen peroxide (H₂O₂), hydroxyphosphatidylcholine (LPC), and 4-hydroxy-2-nonenal (HNE) and...