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EFFECT OF TRANSFORMING GROWTH FACTOR-α ON COLLAGEN PRODUCTION AND SECRETION IN CARDIAC FIBROBLASTS

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Objective. In vitro, the effect of transforming growth factor-α (TGF-α) on collagen production and secretion was investigated in cardiac fibroblasts obtained from normal male adult rats.

Methods. Cardiac fibroblasts from passage 2 were cultured to confluency and incubated in the presence of TGF-α in a concentration range of 0.1 to 100 ng/ml, for 3 days in Dulbecco’s MEM medium. The collagen production and secretion were estimated by ^1H-Proline incorporation; the non-collagen protein (NCP) production and secretion were also calculated.

Results. TGF-α induced a dose-dependent increase in collagen production and secretion from 0.1 to 100 ng/ml (figure). NCP secretion was only moderately increased by high concentrations of TGF-α; these increases averaged, respectively, 6.4 ± 6.6 % at 0.1 ng/ml; 4.2 ± 8.3 % at 1 ng/ml; 8.0 ± 7.2 % at 5 ng/ml; 22.8 ± 10.4 % at 10 ng/ml; 20.0 ± 11.1 % at 50 ng/ml and 10.4 ± 4.6 % at 100 ng/ml NCP production increased only at 50 and 100 ng/ml TGF-α by 39.5 ± 9.4 % and 43.9 ± 6.9 %, respectively.

Conclusion. Our data show that transforming growth factor-α induces a dose-dependent increase in collagen production and secretion in cardiac ventricular fibroblasts, while the non-collagen production and secretion are only moderately affected.

Key Words: Transforming growth factor-α, Fibroblasts, Collagen

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INCREASED DNA DAMAGE WITH DECREASED ACTIVITY AND EXPRESSION OF DNA REPAIR ENZYME hMYH IN HUMAN MYOCARDIUM WITH SEVERE HEART FAILURE

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Enhanced production of oxygen free radicals may play an important role in cardiovascular diseases. Among the different oxidative-damage DNA products, 8-oxo-7,8-dihydrodeoxyguanine (8-oxoG) is the most stable and deleterious adduct. Human MutY homolog (hMYH) is an important repair protein to protect cell from oxidative DNA damage. DNA damage in cardiomyocytes may significantly contribute to congestive heart failure (CHF). However, there is no information regarding the 8-oxoG generation and the expression and activity of hMYH in human myocardium. Therefore, the current study is designed to determined the levels of 8-oxoG and hMYH in human atrial and ventricular myocardium which obtained from normal subjects (n = 4) and CHF patients (n = 5) during cardiac transplantation. The DNA damage such as 8-oxoG generation was determined by staining method. The expression of hMYH was determined by Western blotting and immunohistochemical staining (IHCs). The hMYH activity was measured by gel-retardation binding assay. Cardiac apoptotic level was determined by TUNEL staining and DNA gel analysis. 8-oxoG generation was markedly increased in CHF ventricular tissue combined with significantly decreased hMYH protein level. IHCs illustrated that hMYH was located in nuclear and mitochondria of cardiomyocytes. Furthermore, hMYH activity was significantly decreased (20-fold) in CHF patients compared with normal subjects. TUNEL, DNA gel analysis and IHCs study demonstrated that apoptotic level was significantly increased with markedly enhanced p53 expression in cardiomyocytes with severe CHF. The current study first time demonstrates that 8-oxoG generation was significantly increased and hMYH protein level and activity were markedly decreased in human myocardium with severe CHF. Furthermore, cardiac apoptotic level and p53 expression were significantly increased in CHF myocardium. These data suggest that hMYH plays an important pathophysiological role in cardiomyocyte DNA damage, repair mechanisms, and the process of apoptosis in CHF.

Key Words: Heart failure, 8-oxoG, DNA damage

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REDOX SIGNALING AND VASCULAR REACTIVITY IN BARTTER’S AND GITELMAN’S SYNDROMES (BS/ GS)

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BS/GS are characterized by reduction of G protein-mediated cell signaling (reduced Goq gene expression, intracellular Ca ++ and IP 3 concentration and PKC activity), and an up regulation of NO system (increased ecNOS mRNA production, NO metabolites and cGMP)(Calo L et al, Crit Rev Clin Lab Sci 2000, in press). These abnormalities contribute to BS/GS vascular hyporeactivity and normo-hypotension. BS/GS are protected from oxidative stress-induced endothelial damage (reduced LDL susceptibility to oxidation, reduced volatile LDL oxidation products) (Calo L et al, J Hypert 16:1001-8,1998) notwithstanding high level of angiotensin II which increases O 2 - and other ROS and induces oxidative stress signaling leading to vascular growth, remodeling and atherogenesis.

The present study investigates the redox signaling in 2 BS and 7 GS genetically characterized using a molecular biology approach. It evaluates the gene expression of p22phox, a NADPH oxidase subunit, of heme oxygenase-1 (HO-1), protective toward oxidative stress, and of TGF β major profibrotic cytokine in monocytes by PCR using specific primers. mRNA production was quantified by densitometric analysis using the ratio with β actin. Plasma anti oxidant power (AOP) (ELISA) and peroxynitrates (HPLC as nitrotyrosine) (OONO - ) were also evaluated. p22phox mRNA (0.53 ± 0.05 vs 0.35 ± 0.08, p = 0.006) and TGFβ mRNA (0.82 ± 0.07 vs 1.15 ± 0.25, p = 0.04) were reduced in BS/GS vs healthy controls (C), while HO-1 mRNA (0.88 ± 0.07 vs 0.78 ± 0.11, p = 0.04) and AOP (3.27 ± 0.95 mmol/L vs 1.05 ± 0.16, p = 0.0001) were increased. OONO - was undetectable both in BS/GS and C.

These data establish that protective mechanisms against oxidative stress, remodeling and atherogenesis, such as HO-1 and NO systems are up regulated in BS/GS. HO-1 induced production of vasodilatory CO may contribute, together with NO, to BS/GS vascular hyporeactivity and normo-hypotension. Finally, these data in BS/GS represent the mirror image of derangements involved in hypertension and may contribute to the understanding of mechanisms involved in the pathophysiology of hypertension.

Key Words: Oxidative stress, Vascular reactivity, Bartter’s and Gitelman’s Syndrome