P10 TARGETED STERICALLY STABILIZED LIPOSOMES FOR DIAGNOSTIC IMAGING OF ATHEROSCLEROTIC PLAQUES

R. Prassl[1], G. Almer[2], M. Saba-Lepek[1], D. Frascione[1], J. Kellner[3], A. Gries[3], C. Diwoky[4], R. Stollberger[4], H. Mangge[2]. *[1]Institute of Biophysics and Nanosystems Research, Austrian Academy of Sciences, [2]Clinical Institute for Medical and Chemical Diagnosis, [3]Institute of Physiology, Medical University Graz, [4]Institute of Medical Engineering, University of Technology Graz, Graz, Austria*

We have designed polyethylene glycol (PEG-) coated (sterically stabilized) liposomes to carry high payloads of imaging reagents such as paramagnetic contrast agents (Gd-based), ironoxide nanoparticles or optical active compounds (e.g. fluorescent dyes) to cells with the goal to improve non-invasive molecular imaging modalities by specific targeting. To target atherosclerotic plaques we have covalently coupled selected biomarkers to the distal ends of the PEG-chains, which are located at the surface of the liposomes. These functionalized liposome constructs were characterized by photon correlation spectroscopy, modified native gel electrophoresis and Western Blot. As targeting sequences we have chosen interleukin-10 (IL-10), the globular domain of adiponectin (gAcrp30) and anti-LOX-1 mAB. These biomarkers bind to atherosclerotic aortas of ApoE-deficient mice as shown by *ex vivo* imaging using confocal laser-scanning microscopy (CLSM). For the ligand-conjugated liposomes we found a pronounced signal enhancement ex vivo, whereas no signal was detected in less injured aortic surfaces or in arteries of WT-mice. With IL-10-targeted liposomes we already observed a strong *in vivo* staining signal with CLSM. Now, Gd-DTPA-lipid and ultra small ironoxide nanoparticle (USPIOs) containing targeted liposomes are established to improve image sensitivity for *in vivo* magnetic resonance imaging (MRI). A contrast enhancement in terms of T_1-relaxivity or in hypotense T_2-signals could be achieved for paramagnetic or magneto-liposomes, respectively. In conclusion, the combinatory use of specific anti-inflammatory biomarkers as targeting sequences and multiple or high payloads of contrast agents within one liposome particle opens the opportunity for early recognition, differentiation and visualization of unstable vulnerable atherosclerotic plaques by imaging.

P11 WHHLMI RABBIT IS AN ANIMAL MODEL FOR ANGINA AND/OR CORONARY SPASMS

T. Kobayashi[1], T. Ito[1], S. Yamada[1], N. Hirayama[1], K. Hirata[2], T. Ishida[2], M. Shiomi[1]. *[1]Institute for Experimental Animals, [2]Division of Cardiovascular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan*

Purpose: Coronary spasms are one of the important causes of myocardial ischemia. However, the mechanisms of coronary spasms and the highly susceptible regions are still unknown because of the lack of suitable animal models for the disease. Therefore, we are trying to develop an animal model for coronary spasms and/or angina.
Methods: Coronary atherosclerosis and myocardial infarction-prone Watanabe heritable hyperlipidemic (WHHLMI) rabbits were used in this study. Under anesthesia with intravenous injection of ketamine plus midazolam, WHHLMI rabbits were administered intravenously with serotonin, ergonovine, dobutamine, norepinephrin, or angiotensin-II. ECG and blood pressure at the femoral artery were recorded during the experiment. After examination of ECG, the coronary atherosclerosis was examined histopathologically.
Results: The blood pressure was raised by more than 50 mmHg with intravenous injection of each drug. ECG documented depression of the ST segment, reduction of R-wave amplitude, or T-inversion. Ventricular arrhythmia was also observed after bolus injection of serotonin or ergonovine in combination with the perfusion of dobutamine, norepinephrin or angiotensin II. These ECG changes were correlated with the degree of coronary atherosclerosis.
Conclusions: WHHLMI rabbits will be an useful animal model for angina in which coronary spasm and subsequent myocardial ischemia can be pharmacologically evoked in vivo.

P12 PREVENTION OF ACTIVATED MACROPHAGE-INDUCED ADHESION MOLECULES BY CHLORELLA EXTRACT IN ENDOTHELIAL SEVC CELLS

M.-F. Shih[1], J.-Y. Cherng[2]. *[1]Pharmacy, Chia-Nan University of Pharmacy & Science, Tainan, [2]Biochemistry & Chemistry, National Chung Cheng University, Chia-Yi, Taiwan R.O.C.*

The inflammatory response in large vessels involves the up-regulation of vascular adhesion molecules such as vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM-1), and E-selectin. Inflammatory cytokines such as TNF-α, IL-1, or IL-6 are thought to play important roles in the development of atherosclerosis. Chlorella has been shown to lower high fat diet-induced atherosclerosis. In addition, we have previously shown that partial purified lipophilic chlorella extract (PPLEC) possess strong anti-inflammatory effect. The aim of this study is to investigate the possible preventing role of PPLEC on pro-inflammatory cytokine-induced expression of vascular adhesion molecules.

Endothelial cells (SEVC cell line) were treated with conditioned culture media (normal culture media contains 50% of LPS-activated macrophage culture media, in which there contained TNF-α, IL-1, and IL-6) with or without high (0.5 mg/ml) or low (0.125 mg/ml) dose of PPLEC extracts. Indomethacin (0.25 μM) was used as a positive control. Production of VCAM-1, ICAM-1 and E-selectin was measured by ELISA assay kits.
Production of ICAM, VCAM or E-selectin was all increased by additional RAW culture media in SEVC cells. The induction of E-selectin and ICAM was significantly prevented by both high and low doses of PPLEC or indomethacin treatment. However, the prevention of VCAM production was only seen in high dose of PPLEC.
PPLEC not only possess anti-inflammatory effect (previous study) but also prevents pro-inflammatory cytokines-induced adhesion molecule production in endothelia. These data indicate that PPLEC can be a potential material to develop in preventing chronic inflammatory-related diseases.

P13 CILOSTAZOL AMELIORATES METABOLIC ABNORMALITIES IN A DB/DB MOUSE MODEL OF TYPE 2 DIABETES VIA ACTIVATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORγ TRANSCRIPTION

S.Y. Park, K.W. Hong, W.S. Lee, C.D. Kim. *Medical Research Center for Ischemic Tissue Regeneration; Department of Pharmacology, Pusan National University, Gyeongnam, Republic of Korea*

This study evaluated the *in vivo* efficacy of cilostazol to protect a db/db mouse model of Type 2 diabetes against altered metabolic abnormalities and pro-inflammatory markers via activation of PPARγ transcription. Eight-week old db/db mice were treated with cilostazol or rosiglitazone for 12 days. Cilostazol significantly decreased plasma glucose and triglyceride levels, as did rosiglitazone, a PPARγ agonist. Elevated plasma insulin and resistin levels were significantly decreased by cilostazol, and decreased adiponectin mRNA expression was elevated along with increased plasma adiponectin. Cilostazol significantly increased both adipocyte fatty acid binding protein (aP2) and fatty acid transport protein (FATP-1) mRNA expressions with increased glucose transport 4 in the adipose tissue.
Cilostazol and rosiglitazone significantly suppressed pro-inflammatory markers (superoxide, TNF-α and vascular cell adhesion molecule-1) in the carotid artery of db/db mice. In *in vitro* study with 3T3-L1 fibroblasts, cilostazol significantly increased PPARγ transcription activity, as did rosiglitazone. The transcription activity stimulated by cilostazol was attenuated by KT5720, a cAMP-dependent protein kinase inhibitor, and GW9662, an antagonist of PPARγ activity, indicative of implication of PI3-k/Akt signal pathway. These results suggest that cilostazol may improve insulin sensitivity along with anti-inflammatory effects in Type 2 diabetic patients via activation of both cAMP-dependent protein kinase and PPARγ transcription.

P14 PREVENTION OF DOCA/SALT HYPERTENSION-INDUCED RAT RENAL INJURY BY ANTIOXIDANT THERAPY

M. Kadkhodaee[1], B. Seifi[1], S.M. Karimian[1], M. Zahmatkesh[1], E. Bakhshi[2]. *[1]Physiology, [2]Biostatistics, Tehran Medical Sciences University, Tehran, Iran*

Introduction: Hypertension is a major cardiovascular risk factor and a contributor to End Stage Renal Disease. This study examined whether the antioxidant therapy with vitamin-E or C, could modify renal damage and high blood pressure in DOCA-salt induced hypertension.
Methods: One week after uninephrectomy, rats in the DOCA-salt group treated 5 times a week with DOCA suspended in oil, which were administered subcutaneously for 4 weeks (25 mg/kg). 1% NaCl + 0.2% KCl were added to their water for drinking. Sham rats were received oil, 5 times a week, subcutaneously. In the two other groups vitamin-E (200 mg/kg/day/gavage) or vitamin C (200 mg/kg/day/gavage) were co-administered with DOCA-salt for 4 weeks. Systolic blood pressure (SBP) was measured by the tail-cuff method. Levels of renal antioxidants, renal damage indices and histological changes were studied in all groups.
Results: DOCA-salt treated rats exhibited marked increase in blood pressure compared to that in sham group (183.57 ± 6.24 vs 109.28 ± 2.97 mmHg). Levels of urinary N-acetyl glucoaminidase) NAG and protein excretion were significantly increased. Decreased renal reduced glutathione (GSH) contents and ferric reducing ability of plasma (FRAP) were demonstrated in DOCA-salt treatment as well as histological changes. Treatment with vitamin C or vitamin-E for 4 weeks preserved the renal antioxidant levels and prevented renal damage and elevation of blood pressure in the DOCA-salt treatment.
Conclusions: Antioxidant therapy decreased renal damage in DOCA-salt treated rats. These data suggests a role for oxidative stress in the development of nephropathy in DOCA-salt hypertension.