For ex vivo data, cardiac apoptosis was induced by administration of doxorubicin (20 mg/kg i.p.). Heart sections obtained four days after doxorubicin displayed a more than 3 fold increase in caspase-3 activity, and a marked increase in the fluorescent signal for cleaved CP220, which colocalized with that of the antibody against cleaved caspase-3. These data demonstrate the capacity of these novel probes to detect apoptosis in vitro and ex vivo, allowing us to plan tests of in vivo detection of cardiac apoptosis.

E002
CIRCULATING MICROPARTICLES FROM A RAT MODEL OF PULMONARY ARTERIAL HYPERTENSION INDUCE ENDOTHELIAL DYSFUNCTION

S. TUAL-CHALOT 1, C. GUIBERT 2, J.-P. SAVINEAU 2, R. ANDRIANTSITOHAINA 1, M.-C. MARTINEZ 1
1 CNRS UMR 6214-Inserm 771, Angers, France
2 Inserm 885, Bordeaux, France

Pulmonary arterial hypertension (PAH) is a rare and severe disease characterized by an increase of pulmonary vascular resistance and right heart failure. Chronic hypoxia induces PAH, which is accompanied by functional (endothelial dysfunction, increased vasoconstriction) and structural (thickening of media) changes in pulmonary arteries. However, the mechanisms of these alterations remain unsolved. Among biological hallmark of this disease, level of circulating microparticles (MPs), small vesicles of plasma membrane released during cell activation and apoptosis, is increased in PAH patients. Although MPs can act as biological vectors of endothelial dysfunction, their role in PAH are not elucidated yet. We studied circulating MP effects on endothelial function during hypoxic PAH. Male Wistar rats were exposed or not to chronic hypoxia (3 weeks, 0.5 atmosphere) and normoxic or hypoxic MPs were isolated from peripheral blood exposed or not to chronic hypoxia (3 weeks, 0.5 atmosphere) or no SS (static) for 24 hours. EMPs were isolated from the culture medium and characterized by annexin V labeling using flow cytometry analysis. HUVECs exposed to high SS for 24 hours emitted 4 fold less AnnexinV+ EMPs compared to static (p<0.001) and 3 fold less compared to low SS (p<0.01) conditions. This flow-dependent EMP shedding was associated with the presence of 1.7±0.05, 3.75±0.1, 0.4±0.06 of TUNEL positive HUVECs in static, low and high conditions, respectively. Treatment of HUVECs with L-NAME (10-6M) significantly increased EMPs in all conditions when compared to untreated cells. Similar EMPs increase was obtained when cells were treated with KTS720, a PKA inhibitor (10-6M) under low and high flow. We further characterized EMPs by determining their surface expression of ICAM-1. ICAM-1 expression on EMPs was significantly increased in low SS and inhibited by high SS when compared to static conditions. Adhesion assays with EMPs-stimulated HUVECs (24hours) increased U937 cells capacity to attach in low SS-EMPs exposed to HUVECs compared to static and high SS EMPs stimulations. This effect was abolished with LFA-pretreated U937 before adhesion suggesting the pro-adhesive properties of low SS-EMPs bearing ICAM-1 at their surface.

Altogether, these findings indicate that high SS decreased the thrombotic and adhesive properties of EMPs, which might in part explain their anti-atherogenic effects, whereas low SS induced the shedding of prothrombotic, ICAM-1 positive EMPs, suggesting a novel way by which low SS might affect atherosclerosis.

E003
SHEAR STRESS MODULATES ENDOTHELIAL MICROPARTICLES SHEDDING

B. RAMKHELAWON 1, S. LEHOUX 1, C. DEVUE 1, P.-E. RAUTOU 1, A. TEDGUI 1, C. BOULANGER 1
1 Inserm U689, Paris, France

Plasma levels of endothelial microparticles (EMPs) are markers of cardiovascular diseases and contribute to the pathogenesis of atherosclerosis. Laminar shear stress (SS) protects against plaque formation contrarily to oscillatory and low SS. We thus investigated whether different flow patterns (laminar, low or static) could affect EMP release.

HUVECs were subjected to high and low SS (15, 1.5 dynes/cm²) or no SS (static) for 24 hours. EMPs were isolated from the culture medium and characterized by annexin V labeling using flow cytometry analysis. HUVECs exposed to high SS for 24 hours emitted 4 fold less AnnexinV+ EMPs compared to static (p<0.001) and 3 fold less compared to low SS (p<0.01) conditions. This flow-dependent EMP shedding was associated with the presence of 1.7±0.05, 3.75±0.1, 0.4±0.06 of TUNEL positive HUVECs in static, low and high conditions, respectively. Treatment of HUVECs with L-NAME (10-4M) significantly increased EMPs in all conditions when compared to untreated cells. Similar EMPs increase was obtained when cells were treated with KTS720, a PKA inhibitor (10-6M) under low and high flow. We further characterized EMPs by determining their surface expression of ICAM-1. ICAM-1 expression on EMPs was significantly increased in low SS and inhibited by high SS when compared to static conditions. Adhesion assays with EMPs-stimulated HUVECs (24hours) increased U937 cells capacity to attach in low SS-EMPs exposed to HUVECs compared to static and high SS EMPs stimulations. This effect was abolished with LFA-pretreated U937 before adhesion suggesting the pro-adhesive properties of low SS-EMPs bearing ICAM-1 at their surface.

Altogether, these findings indicate that high SS decreased the thrombotic and adhesive properties of EMPs, which might in part explain their anti-atherogenic effects, whereas low SS induced the shedding of prothrombotic, ICAM-1 positive EMPs, suggesting a novel way by which low SS might affect atherosclerosis.

E004
LES MICROPARTICULES POSITIVES POUR LE CD11B : UN MARQUEUR PREDICTIF DES EVENEMENTS RECURRENTS CHEZ LES PATIENTS CORONAIRES

D. FAILLE 1,2,3, C. FRERE 1,2,3, T. CUISSET 4, J. QUILICI 4, P.-J. MORO 4, P.-E. MORANGE 1,2,3, I. JUHAN-VAGUE 1,2,3, J.-L. BONNET 4, M.-C. ALESSI 1,2,3
1 Inserm, UMR 626, Marseille, France
2 Aix-Marseille Université, Faculté de Médecine, Marseille, France
3 Laboratoire d’Hématologie, CHU de la Timone, Marseille, France
4 Département de Cardiologie, Marseille, France

Background — La thrombose et l’inflammation sont impliquées dans l’initiation et la progression de la lésion athéromateuse. Ainsi, de nombreuses cellules du compartiment vasculaire jouent un rôle clé dans la pathogénèse de la maladie coronarienne et ses complications. Déterminer précisément l’état d’activation de ces différents types cellulaires pourrait contribuer à identifier les patients coronariens à haut risque d’événements récurrents.