

PO1-14

Enhanced procoagulant activity of monocytic leukemia cell lines and microparticles

Hudak R.¹, Deak I.¹, Szabo Gal G.¹, Beke Debreceni I.¹, Antal-Szalmas P.¹, Kappelmayer J.¹

¹*University of Debrecen, Department of Laboratory Medicine, Debrecen, Hungary*

Aims: In hematological malignancies the expression of haemostatic proteins by tumor cells e.g. tissue factor (TF) production, microparticle (MP) formation, as well as chemotherapy contribute to the enhanced procoagulant activity. We tested the procoagulant activity of monocytic leukemia cell lines and investigated whether entities that contribute to early relapse in AML would affect the procoagulant characteristics of these cells. Thus, cell lines with different FLT3 internal tandem duplication (ITD) mutation status and resistant and sensitive FLT3-ITD heterozygous cell lines were studied.

Methods: All cell lines were grown in RPMI-1640 medium, supplemented with 15% fetal bovine serum. As negative control, normal human monocytes separated by magnetic beads were used, while the positive control was the NB4 acute promyelocytic leukemia cell line. Microparticle separation was performed with multiple high-speed centrifugation. Thrombin generation test (TGT) was used to determine the procoagulant potential of monocytes, cell lines and the microparticles. MP number, TF and phosphatidyl-serine (PS) expression were examined by flow cytometry.

Results: There was no difference in PS expression of cell lines (1-2%), but the THP-1 cell line showed the highest TF expression followed by the MV4-11 and MOLM-13 cells (73%, 21% and 4% respectively). All three cell lines displayed a significantly faster thrombin generation compared to normal human monocytes (lagtime: 3.9-4.7 min. versus 14.9±2.5 min). Factor VII deficient plasma and anti-tissue factor antibody pretreatment abolished this difference, while FXII deficient plasma did not affect the speed of thrombin generation but resulted in an attenuated thrombin formation. In addition we found, that MPs generated by these cells also contribute to the procoagulant activity. The FLT3-ITD mutation status did not influence the procoagulant activity of these cell lines. Resistant MOLM-13 cell lines, however generated a decreased amount of thrombin compared to sensitive cells.

Conclusions: Non-activated human monocytes express TF on their surface but this is functionally inactive. Monocytic leukemia cell lines and their MP exhibit significantly higher procoagulant activity compared to normal monocytes and this effect can be modulated by the development of chemoresistance.

PO1-15

Heparins and fucoidans inhibit the CXCR4 / 7 signalling axis in lymphoma cells by interaction with the chemokine CXCL12

Alban S.¹, Liewert I.¹, Schneider T.¹, Ehrig K.¹

¹*Christian-Albrechts University of Kiel, Pharmaceutical Institute, Kiel, Germany*

Introduction: Chemokines mediate leukocyte trafficking during homeostasis and inflammation and are essential for linking innate and adaptive immunity. The chemokine CXCL12 is the main factor to home hematopoietic progenitor cells to the bone marrow but it also plays an important role in cancer cell proliferation, invasion and metastasis. Therefore, blocking of this signalling axis is a promising strategy for tumor therapy [1-3]. Heparins are known to interact with CXCL12 and various other chemokines and they also exert antimetastatic effects in vivo.

Aims: The aim of this study was to elucidate whether also fucoidans from brown algae interact with CXCL12. Furthermore, the potencies of fucoidans and heparins concerning the inhibition of CXCL12-induced activation of CXCR4 and CXCR7 should be compared.

Methods: The test compounds were the fucoidans from *Saccharina latissima* (S.l.-SP) and *Fucus vesiculosus* (F.v.-SP) as well as unfractionated heparin (UFH) and the low molecular weight heparin tinzaparin. The approved CXCR4-analogue plerixafor (AMD3100, Mozobil™) served as reference compound. CXCR4 and CXCR7 activation in a human Burkitt's lymphoma cell line was monitored by flow cytometry via receptor internalization upon ligand binding. The binding of sulfated glycans to CXCL12 was determined with a competitive