

ORAL COMMUNICATIONS

Antithrombotics and Fibrinolytics in Cancer Survival**OC-01****MODIFIED HEPARINS AS POSSIBLE INHIBITORS OF METASTASIS**

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Hematogenous metastasis of cancer cells is a cascade of events involving many factors which allow tumor cell intravasation into the bloodstream, evasion of innate immune surveillance, interaction with blood cells and vascular endothelial cells of distant organs, and extravasation in these tissues. Malignancy of carcinomas is characterized by enhanced migration, degradation of extracellular matrix, remodeling and/or alterations of integrins or cell adhesion molecules and finally, altered cell-surface glycosylation. Blood born carcinomas interact with platelets and leukocytes thereby forming tumor cell emboli which arrest in capillaries of distant tissues. Cancer progression is often accompanied by higher incidence of thrombotic events. While heparin is commonly used in cancer patients with thrombotic events and show improvements of patient's survival rate, its acti-

vity can not be solely ascribed to anticoagulant activity. Heparin is endowed with several biological activities including inhibition of P- and L-selectins, inhibition of heparanases, binding of growth factors etc. Previously, we have shown that platelet-tumor cell aggregation is P-selectin dependent. Attenuation of metastasis was also observed in the absence of L-selectin, thus implicating leukocytes to be actively contributing to this process. Furthermore, injection of unfractionated heparin prior to tumor cells led to attenuation of metastasis. Heparin injection in P-selectin deficient mice had no further effect on metastasis, indicating that heparin inhibits P-selectin mediated interactions. To dissect the metastatic inhibitory effects of heparin, we tested different heparin derivatives for their selectin inhibitory activity. Through selective desulfation, graded N-acetylation and glycol splitting, several heparin derivatives without any anticoagulant activity were prepared. ELISA screening for their ability to inhibit P- and L-selectin binding to tumor cells resulted in identifying two derivatives with comparable selectin inhibitory activity as seen with unfractionated heparin. Initial in vivo experiments in a carcinoma mouse model indicated that heparins without anticoagulant activity attenuated metastasis to the similar extend as seen with heparin. Further analyses are focused on identification of heparin derivatives with selectin inhibitory activities.

Endothelium Targeting and Anticancer Therapies

OC-02

FGF-2 BINDING SITE IS LOCATED IN THE C-TERMINAL CASSETTE OF THROMBOSPONDIN-1

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Background. Thrombospondin-1 (TSP-1) is a major endogenous angiogenesis inhibitor binding with high affinity to several heparin-binding angiogenic factors. We have previously shown that TSP-1 binding to FGF-2 (*Fibroblast Growth Factor-2*) modulates FGF-2 location, bioavailability and function (*Blood* 2003; 102, 4399-4406). We also reported that the FGF-2 binding and scavenging activity is retained by the C-terminal anti-angiogenic 140 kDa fragment of TSP1.

Aim. The present work aims at identifying more precisely the FGF-2 binding site within the TSP-1 molecule.

Materials and methods. In order to define a smaller region involved in FGF-2 binding, we used recombinant domains of TSP-1 spanning the entire 140 kDa fragment with partial overlaps. We analyzed the binding of biotin-labeled FGF-2 to the panel of domains immobilized on plastic substrate and, as a parallel approach, the binding of the biotin-labeled domains to immobilized FGF-2. We also investigated the formation of a complex between biotin-labeled FGF-2 and the recombinant domains when both molecules were in solution, by Western blot analysis. Finally, we studied the affinity of the domains for FGF-2 by surface plasmon resonance on BIAcore.

Results. The FGF-2 binding site is located within a region comprising the third type II repeat, the type III repeats (which represent the calcium binding region of the molecule), and the C-terminal globular region. Binding occurs when FGF-2 and the domain are in solution and when, alternatively, one of them is immobilized. BIAcore analysis confirmed that the affinity of this region for FGF-2 is high and comparable to that of native TSP-1. As previously shown for the native molecule, heparin prevents FGF-2 binding to the domain. Calcium concentration strongly affects TSP-1/FGF-2 binding.

Conclusions. These findings locate the FGF-2 binding site within the carboxyterminal cassette of TSP-1, thus in a region different from the well-known anti-angiogenic type I repeats.

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OC-03

THE HOMOPHILIC BINDING OF JUNCTIONAL ADHESION MOLECULE-C MEDIATES TUMOR CELL-ENDOTHELIAL INTERACTIONS

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The junctional adhesion molecule-C (JAM-C) was recently shown to undergo a heterophilic interaction with Mac-1 integrin mediating interactions between vascular cells in inflammatory recruitments. Here, we presented that JAM-C can also undergo homophilic interaction, which plays a role in tumor cell-endothelial interactions. Recombinant soluble JAM-C in fluid phase bound to immobilized JAM-C as assessed in a purified system. In addition, JAM-C-transfected cells adhered to immobilized JAM-C. The homophilic interaction of JAM-C was mediated by the aminoterminal Ig-domain, but not the carboxyterminal Ig-domain (D2) of the molecule. Single amino acid mutation in RIE motif (E66R) abolished the homophilic interaction of JAM-C. The lung carcinoma cell lines NCI-H522 and NCI-H322M were found to be positive and negative for JAM-C expression, respectively. In contrast to NCI-H322M cells, NCI-H522 cells adhered to immobilized JAM-C. Moreover, NCI-H522 cells adhered to JAM-C-transfected CHO cells, but not to mock-transfected CHO cells or to CHO cells transfected with the JAM-C mutant (E66R). Adhesion of NCI-H522 cells to JAM-C protein or JAM-C-transfected CHO cells was abolished in the presence of soluble JAM-C or the isolated D1. Furthermore, the adhesion of NCI-H522 cells but not of NCI-322M cells to endothelial cells was significantly blocked by soluble JAM-C or the isolated D1. Thus, JAM-C undergoes a homophilic interaction that is mediated by the motif R₆₄I₆₅E₆₆ in the aminoterminal Ig-domain of the molecule. The homophilic interaction of JAM-C can mediate tumor cell-endothelial cell interactions and may thereby be involved in the process of tumor cell metastasis.

Bleeding and Inflammation

OC-04

IDENTIFICATION OF RISK FACTORS FOR CONSUMPTIVE COAGULOPATHY IN PATIENTS WITH NON-M3 ACUTE MYELOBLASTIC LEUKEMIA

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Background. Disseminated intravascular coagulopathy (DIC) is a life-threatening complication of acute myeloblastic leukaemia (AML). This is characterized by the abnormally regulated activation of procoagulant pathways resulting in decreased levels of hemostatic components. DIC is extremely frequent in acute promyelocytic leukemia but other AMLs can present this complication. Nevertheless, there is scarce information concerning incidence and risk factors for DIC in non-M3 AMLs.

Aim. Analyze clinical and biological features of non-M3 AMLs presenting DIC at first induction therapy.

Patients and methods. Between 1990 and mid-2005, 353 consecutive adult patients (median age 56 years, range 13-80) were diagnosed of *de novo* non-M3 AML and received induction therapy in our institution. Clinical records were reviewed for coagulation abnormalities, at diagnosis and during induction phase, in the absence of other recognizable causes. DIC was defined as the presence of at least three of the following coagulation test abnormalities: elevated D-dimers, prolonged prothrombin time, prolonged activated partial thromboplastin time, or hypofibrinogenemia. Univariate and multivariate analyses were performed to assess statistical significance of a number of potential predictors of DIC.

Results. Thirty-one patients (8,8%) fulfilled DIC criteria, 23 at diagnosis and 8 after the beginning of chemotherapy. FAB subtypes were M1 (42%), M4 (26%), M5 (23%), M2 (6%) and M0 (3%). Patients with DIC showed a lower complete remission rate (39% vs 62%) and a higher induction mortality rate (45% vs 17%, $p < 0,001$). Intracranial haemorrhage and acute distress respiratory syndrome were the main causes of death. DIC was associated with ECOG > 1, gingival hypertrophy, FAB subtype (M1/M4/M5 vs others), blasts in peripheral blood (PB) $> 20 \times 10^9/L$, leucocytes $> 50 \times 10^9/L$, creatinine $> 1,3$ mg/dL, urate $> 7,5$ mg/dL, LDH > 600 ui/L and GOT > 50 ui/L ($p < 0,003$). Leukemic cells of patients with DIC showed more frequently expression of CD9, CD56 antigen and lack of CD34 antigen ($p < 0,003$). In multivariate analysis blasts in PB $> 20 \times 10^9/L$, GOT > 50 UI/L, CD34⁻ and CD9⁺ were independent risk factors for DIC.

Conclusions. This study show that DIC is associated with high blast counts in PB and high induction mortality rate. Cytomorphological and immunophenotypical findings can be useful to identify AMLs with high risk of DIC.

OC-05

INCIDENCE, CLINICAL-LABORATORY FEATURES AND MANAGEMENT OF ACQUIRED VON WILLEBRAND SYNDROME AND OTHER ACQUIRED DEFECTS OF HEMOSTASIS IN A COHORT OF 240 PATIENTS WITH CHRONIC LYMPHO-MYELOPROLIFERATIVE DISORDERS

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Background. Acquired von Willebrand Syndrome (AVWS) is a rare bleeding disorder with laboratory findings similar to those for congenital von Willebrand disease. The actual prevalence of AVWS in the general population is unknown because large prospective studies on this syndrome are not available. Retrospective data showed that AVWS is especially frequent in lympho- (LPD) or myeloproliferative (MPD) disorders.

Aims and design of the study. To determine incidence, clinical-laboratory features and management of AVWS and other acquired hemostatic defects, we have sequentially observed for one year our cohort of patients with chronic LPD/MPD. Exclusion criteria were platelet counts $< 70,000/uL$ and any therapies, including non-steroid anti-inflammatory drugs.

Methods. A bleeding severity score derived from a detailed history of 11 symptoms. Screening tests: bleeding time (BT), prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT) and, if prolonged, PT-PTT-TT 50:50 mixing tests. Additional specific tests: FVIII/VWF activities (AVWS/HA); platelet nucleotides (acquired storage pool defects, ASPD); silice clotting time (SCT), Russel viper venom time (RVVT), anticardiolipin antibodies (ACA) for lupus anti-coagulant-antiphospholipid antibodies (LAC/APA).

Results. Among 458, 240 patients satisfied the inclusion criteria, with percentual (%) diagnosis of MGUS (38), ET (38), CLL (7), PV-CML-IMF (7), HD-NHL (5), MDS M (2), MM (2) and amyloidosis (1). **Results are:**

Features	Lymphoproliferative	Myeloproliferative	Total
Case number (%)	122 (51)	118 (49)	240 (100)
Bleeding score (> 10)	30/122 (25)	18/118 (15)	48/240 (20)
Abnormal screening tests	57/122 (48)	22/118 (19)	79/240 (33)
Acquired defects:	21/122 (17)	38/118 (32)	59/240 (25)
1) AVWS	10/122 (8)	12/118 (10)	22/240 (9)
2) ASPD	0/122 (0)	19/118 (16)	19/240 (8)
3) LAC/APA	8/122 (7)	3/118 (3)	11/240 (5)
4) anti FVIII or X inhibitors	3/122 (2)	4/118 (3)	7/240 (3)

In one year, severe mucosal (n=21) and non-mucosal (n=13) bleeds in LPD (n=12) or MPD (10) were treated with DDAVP (n=18), FFP/concentrates (n=4), IVIg (n=10), rFVIIa (n=2).

Conclusions. AVWS and the other acquired hemostatic defects shown here are not so rare (9/16%) and can be severe in LPD/MPD. An early correct diagnosis should improve morbidity and mortality of patients with bleeding complications in chronic LPD/MPD.

Glycosaminoglycans, Cancer and Thrombosis

OC-06

A CONCERT OF ACTIONS CONTRIBUTES TO THE ANTIMETASTATIC ACTIVITY OF THE SEMISYNTHETIC GLUCAN SULFATE PS3

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Background. According to recent clinical trials, treatment with low molecular weight heparins (LMWH) improves the survival of tumor patients, whereby their main mode of anti-metastatic action is still an unsolved question. Since heparins have several disadvantages (e.g. animal origin, complex composition, batch variability, high anticoagulant activity), heparin-like, structurally defined alternatives with an improved action profile may be an interesting option. We have developed the glucan sulfate PS3 (PCT Int. Appl. WO 2002036132), which was shown to exhibit anti-inflammatory, antiangiogenic and potent TFPI-releasing effects.

Aim. The aim of the present study was to investigate the effects of PS3 and heparin (UFH) on mechanisms involved in tumor cell (TC) metastasis and to compare their anti-metastatic activity *in vivo*.

Materials and methods. The selectin-mediated TC (U937, LS180) adhesion was examined in a static microplate (MP) cell-adhesion assay, a flow chamber model and by intravital microscopy. As a model for the interactions of TC with the basement membrane, the adhesion of TC (MDA-MB231) to laminin was used. The influence on the TC-mediated proteolysis was investigated measuring the inhibition of elastin degradation by TC (MCF-7). The inhibition of the ECM degrading hyaluronidase was evaluated by the colorimetric Morgan-Elson reaction. Finally, the anti-metastatic activity was examined in the B16.F10 melanoma lung metastasis model in mice.

Results. PS3 showed to interfere with several processes involved in metastasis. In all the assays, PS3 proved to be superior to UFH. It blocks the P- (IC₅₀ 5 µg/mL) and L-selectin- (IC₅₀ 10 µg/mL) mediated TC adhesion and thus an initial step of their extravasation. It inhibits the TC adhesion to laminin (IC₅₀ 5 µg/mL) and thus impairs the interactions of migrating TC with the basement membrane. In contrast to UFH, PS3 concentration-dependently inhibits the elastolytic activity of TC. In addition, it inhibits hyaluronidase (IC₅₀ 4.5 µg/mL), which is produced by certain TC. In Agreement with the *in vitro* results, PS3 was about ten times more active than UFH in inhibiting lung metastasis formation *in vivo*.

Conclusions. Like heparins the semisynthetic glucan sulfate PS3 represents a multivalent biomodulator suggesting that not a single, but rather the concert of several effects is responsible for the anti-metastatic activity.

Recent Advances on Prevention and Treatment of VTE in Cancer

OC-07

WARP: A NATIONAL RANDOMISED CONTROLLED TRIAL OF THROMBOSIS PROPHYLAXIS WITH WARFARIN IN CANCER PATIENTS WITH CENTRAL VENOUS CATHETERS

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Background. Catheter-related (CVCr) thrombosis is a significant cause of morbidity and mortality in cancer patients. We report the results of a large pragmatic RCT of thrombosis prophylaxis in cancer patients receiving chemotherapy via CVCs.

Aim. To investigate the utility of warfarin and two dosing strategies in the prophylaxis of thrombosis.

Methods. Patients, over 16 years old, receiving chemotherapy via CVCs, with adequate haematological parameters and no contra-indication to, or not currently on warfarin, were randomised according to clinicians' prescribing practices: *Uncertain indication* - randomised to control (no warfarin) vs warfarin (1 mg daily or dose adjusted warfarin (DAW) to maintain INR between 1.5 and 2.0). *Certain indication* - randomised to warfarin 1mg daily vs DAW. The primary outcome was the number of radiologically proven CVCr, symptomatic thrombotic events (STEs). Major bleeding events (MBEs) are reported.

Results. 1,589 patients were randomised. The overall rate of CVCr symptomatic thrombosis was low at 5%. *Warfarin vs Control:* 408 patients were randomised to warfarin (20%, DAW; 80%, 1mg); 403 to control. Warfarin did not decrease CVCr STEs (5% warfarin vs 5% control; odds ratio (OR)=1.04 (95% confidence interval (CI): 0.56, 1.92); *p*>0.99) and was associated with a greater frequency of MBEs (2% vs 0.2% respectively; *p*=0.07). *DAW vs 1 mg Warfarin:* 473 patients were randomised to DAW; 471 to 1mg warfarin. DAW prevented more CVCr STEs than 1mg warfarin (2% vs 7% respectively; OR=0.29 (95%CI: 0.15, 0.59); *p*<0.01) and was associated with a greater frequency of MBEs (3.5% vs 1.6% respectively; *p*=0.13).

Conclusions. There is no apparent benefit in using low dose warfarin for prophylaxis of symptomatic CVC-related thrombosis in patients with cancer. However, if clinicians choose to offer prophylaxis, DAW would seem superior at a cost of an increased bleeding diathesis.