Coagulation, angiogenesis and cancer

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Summary

The relation between cancer and coagulation has been a well known phenomenon for years. The chance that thrombosis occurs is six times higher in cancer patients compared to healthy persons. In the event of metastasis, this chance is even twenty times higher. Both cancer itself and therapy against cancer can induce a pro-thrombotic state. Conversely, evidence is accumulating that activation of the coagulation system contributes to cancer progression and metastasis. In randomized trials, in which efficacy and safety of low molecular weight heparins (LMWHs) for the initial treatment of thrombosis was compared with that of unfractionated heparins (UFH) (both anticoagulants) an unexpected mortality reduction was observed in the sub-group of cancer patients who were treated with LMWH, as compared to UFH recipients. These findings could not be explained by differences in fatal thromboembolic complications or haemorrhages. In following studies, the effects of LMWH compared to placebo on cancer survival in patients were described. The median survival in the group treated with LMWH was higher compared to the placebo group. These clinical results therefore suggest that LMWH interferes with the malignant process and affects survival of patients with cancer.

Chapter 1 of this thesis consists of an introduction on the interaction between cancer and coagulation. First cancer patients have an increased risk to develop thrombosis, and second the coagulation system affects cancer progression and metastasis in which anticoagulants may serve as anticancer therapy. This thesis focuses on three major cancer progression-stimulating factors; platelets, thrombin and angiogenesis (the formation of new blood vessels) and the two way interaction between cancer and coagulation.

Chapter 2 reviews animal studies in which anticoagulants were tested as anticancer drugs to elucidate potential mechanisms by which these agents may affect cancer progression. Anticoagulants seemed to affect the formation of metastasis rather than the growth of the primary tumor. To optimize the effect of anticoagulants data were collected on the type and dose of anticoagulants, duration of exposure and interval between the anticoagulant administration and cancer cell inoculation.

Chapter 3 to 7 describe animal studies that were performed to elucidate the mechanisms for the effects of anticoagulants on cancer survival that we found in clinical studies.

Chapter 3 reviews the validation of a technique, bioluminescence (BLI), for quantitative assessment of tumor load in small animals used in some of our studies. Cancer cells were grafted in mice after transfection with a luciferase gene. To determine tumor load, animals received the substrate agent luciferin intraperitoneally, which then converts into oxyluciferin. Light emitted by oxyluciferin in viable cancer cells was captured noninvasively.
with a highly sensitive CCD camera and correlated with the amount of tumors in the mouse. This technique obviously has advantages compared to the conventional pulmonary metastastic lung or liver metastasis model.

**Chapter 4** shows that the effect of anticoagulants on cancer also depends on the type of cancer. We studied the effects of long-term sub-continuous administration of anticoagulants -using micro-osmotic pumps on colon cancer metastasis in mice. Although this type of administration ensured long term anti Xa levels (a measure for LMWH activity) within the therapeutic range, comparable to that in humans, differences in numbers, size of metastasis, fibrinogen content and vessel density could not be found between treated animals and controls.

In **chapter 5**, we investigated the effects of LMWH and hirudin (a specific anti thrombin inhibitor) on a K1735 melanoma cell line. We also compared the effect of hirudin on three different cancer cells lines. LMWH and hirudin were not able to inhibit metastasis development after inoculation of K1735 melanoma cancer cells. Hirudin inhibited metastasis of the B16 melanoma cancer cells completely but did not affect metastasis of CT 26 colon carcinoma cells. It was concluded that the antimetastatic effects of anticoagulants are dependent on the cancer cell type. CD24 expression, a surface protein, on cancer cells and possibly PAR-1 expression seemed to be required.

Genetic predisposition may be involved in the susceptibility to develop metastasis. In **chapter 6**, we studied the effects of the factor V Leiden mutation (higher risk to develop thrombosis) and the factor VIII (bleeding risk) mutation on the development of experimentally induced metastasis in mice. Indeed, factor V Leiden mice developed more metastases, in contrast to factor VIII deficient mice who were protected against lung metastasis. Overall, these data showed that a congenital susceptibility to either bleeding or thrombosis modifies the metastatic capacity of cancer cells and suggests that procoagulant phenotypes are a risk factor for tumor metastasis.

Previous experimental studies showed that hirudin inhibited metastasis most efficiently. In **chapter 7** we aim to assess the antimetastatic effect of long-term treatment with different specific anti thrombin inhibitors in a mouse pulmonary melanoma metastasis model. Long-term administration of ximelagatran, an oral thrombin inhibitor, and hirudin both increased the number of lung metastasis compared to a control group. Long-term thrombin inhibition seems detrimental and increased pulmonary lung metastasis. Therefore, antithrombotic therapy may promote tumor metastasis suggesting that thrombin inhibition in cancer patients should be pursued with great care.
As described above, several studies have recently shown an anticancer activity of LMWH, including the MALT study. In chapter 8 we measured the levels of the cytokines interleukin-6 and -10 and P-selectin in blood from patients included in the MALT study. In the MALT study patients were randomized between LMWH and best supportive care. We found that increased interleukine-10, interleukin-6 and P-selectin levels predicted a poor outcome in patients with advanced stage cancer. In particular, high interleukin-10 predicted a twofold increase in the risk of dying, even after adjustment for other prognostic markers. The prolongation in survival observed with LWMH therapy was not explained by an effect of LWMH on interleukin-10, interleukin-6 and P-selectin circulating levels.

In chapter 9, we investigated in a randomized placebo-controlled double blind trial the efficacy and safety of thromboprophylaxis with subcutaneous LMWH in patients with haematological malignancies with central venous catheters. The frequency of venographically proven catheter related thrombosis was low in both study groups and was not further reduced by LMWH administration. The thromboprophylaxis regimen used appeared to be safe with no differences in catheter-related infections or bleeding events between the two study groups.

Vascular endothelial growth factor (VEGF) is an important angiogenic factor. The role of circulating VEGF levels as marker for prognosis and response prediction is controversial. The heterogeneity in methods of VEGF measurements is one of the factors that contribute to the different outcomes in the various studies. The aim of the study in chapter 10 was to determine the effect of different blood collection protocols on the measurement of circulating VEGF and the impact of VEGF release from platelets by in vitro platelet activation. Our findings suggest that true freely circulating VEGF levels are not elevated in most cancer patients. The previously reported elevated plasma VEGF levels in cancer appear to be due to release from activated platelets with increased VEGF content during the blood harvest procedure. Only in patients with renal cell cancer, a cancer type characterized by excessive VEGF production due to a specific genetic defect, circulating VEGF was truly elevated.

Chapter 11 studies the change in angiogenic biomarkers during sorafenib treatment and correlations with clinical outcome. Several preclinical studies demonstrated that changes in angiogenic biomarker levels in plasma during VEGFR targeted therapy is a class effect, is tumor independent, and may reflect optimal VEGFR inhibition, but cannot be considered as a predictive marker for tumor response or clinical benefit. The absence of correlations between angiogenic biomarker changes during sorafenib treatment and clinical outcome are in agreement with these preclinical observations.
References

