Tissue procoagulant activity may be important in sustaining metastatic tumour growth

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There is strong evidence for an association between the haemostatic system and malignancy. Thus, cancer may adversely affect the host coagulation system while the haemostatic system may play a role in the development of both primary and metastatic tumours. Metastatic growth is not dependent simply on haemodynamic factors, and properties of both the tumour cell and host organ are important determinants of the site of metastatic growth. Previous studies have demonstrated that some organs are preferred sites for metastasis while others are less preferred or resistant. We have measured the procoagulant activity (PCA) of normal rat and human tissues and correlated the results with the previously reported ability of these organs to support metastatic tumour growth. In addition, we determined changes in PCA in rat tissues during oral anticoagulant therapy, and following colonic anastomosis and partial hepatectomy, procedures which are known to affect experimental metastasis. In both rat and human studies, organs which are preferred sites for metastasis had significantly higher PCA than non-preferred organs (P < 0.001). The PCA of adrenal, lung and colon was significantly reduced by administration of warfarin (P < 0.001). PCA was significantly (P < 0.001) increased in both colonic anastomoses and regenerating liver and followed a time course similar to that of the enhanced tumour growth usually seen in these situations. Although the exact source of the procoagulant activity remains to be determined, the results suggest that there is a broad correlation between tissue PCA and the ability of a tissue to support metastatic tumour growth.

Keywords: metastasis, blood coagulation, procoagulants, oral anticoagulants

Introduction

In human cancer, the site of early metastatic growth is largely the result of haemodynamic factors. The venous drainage of the primary tumour determines the organ which first receives the malignant cells — the ‘organ of first encounter’ [1]. Thus, tumours with a portal venous drainage spread initially to the liver, while those with a systemic drainage progress to the lungs [2]. The later patterns of spread and tumour dissemination in animal systems are not explained by this simple model. Once cells have entered the arterial system the pattern of metastasis is similar for almost all carcinomas [1] but the factors which govern successful metastatic growth are not known. Over 100 years ago Paget [3] noted that not all tissues are similarly receptive to metastatic tumour growth. Indeed, given a dose of viable tumour in proportion to their percentage of cardiac output, some organs are unexpectedly frequent sites of tumour growth [4]. Thus, a ranking of ‘fertility’ for tumour growth can be constructed in which adrenal and lung are good ‘soils’, while colon and liver are relatively poor [5]. The ability of the latter organs to support tumour growth can, however, be enhanced by trauma [6] and the process of tissue regeneration [7].
As noted above, the factors which influence and control metastasis are poorly understood. The common occurrence of tumour-associated fibrin has stimulated many studies of the interaction of malignant cells with the blood coagulation system [8]. The association of abnormal haemostasis and malignant disease is now well established [9-11] and many authors have documented the increased frequency of thrombosis in patients with malignant tumours [12-14]. Using sensitive laboratory tests, coagulation abnormalities can be detected in the peripheral blood of around 90% of cancer patients [15]. Both extra- and intravascular coagulation are assumed to be at least partly due to procoagulant substances produced by tumour cells and tumour-associated macrophages [16]. However, little work has previously been performed to determine how the procoagulant activity of normal tissues varies between different organs and how this might influence their fertility for malignant growth. The aim of the present study was therefore to explore how the fibrin-forming capacity of various rat tissues, as measured by their ability to activate coagulation factor X, correlates with the reported ability of these tissues to support metastatic tumour growth. This relationship was assessed in four situations: (1) in normal rat organs and in human tissues obtained at post-mortem, (2) in organs from normal rats on oral anticoagulant therapy, (3) in normal rat colon following colonic anastomosis and (4) in normal rat liver regenerating after partial hepatectomy.

Materials and methods

Hooded Lister rats weighing 200-250 g were used in all experiments. The preparation and assay of tissue samples were the same in each protocol and are described in detail below.

Sample preparation

Tissue samples were removed, weighed and snap-frozen in liquid nitrogen. Samples were then homogenized by cryofragmentation in a Braun Mikrodismembrator (F. T. Scientific Instruments Ltd, Tewkesbury, UK). The powder was suspended in a 10-fold (w/v) volume of assay buffer (0.05 M Tris-Cl, pH 7.8). The resultant homogenates were then centrifuged at 10,000 g for 2 min and the supernatant removed and stored at -20°C until required for assay.

Procoagulant assay

Procoagulant activity (PCA) was determined by a specific chromogenic substrate assay adapted from Colucci et al. [17]. Wells of a flat-bottomed microtitre plate contained 40 µl of assay buffer, 20 µl of calcium chloride (0.025 M), 20 µl of tissue homogenate and 20 µl of purified human factor X (Sigma Chemical Co., Poole, UK). A blank well for each sample consisted of the above mixture with buffer in place of factor X. The plate was incubated at 37°C for 30 min. The generation of factor Xa was then terminated by addition of 100 µl of 7.5 mM EDTA in assay buffer. Forty µl of chromogenic substrate CBS 31.39 (Diagnostica Stago Ltd, Asnières, France) was then added and the incubation continued for a further 45 min. This reaction was finally stopped with 50 µl of glacial acetic acid. The optical density of each well was then read at 410 nm in a Dynatech MR700 plate reader. PCA was expressed in absorbance units (corrected for the sample blank) per milligram of homogenate protein. Protein concentrations were measured with a commercially available dye-binding assay (Pierce Chemical Co., Chester, UK).

Procoagulant activity of normal rat organs

Normal Hooded Lister rats were killed under ether anaesthesia. Organ samples were immediately removed and assayed for PCA. The types of organ studied, and the number of assays performed are detailed in Table 1.

The procoagulant activity of post-mortem human tissue

Samples of human organs (Table 2) from subjects undergoing post-mortem, who had no evidence of malignant disease, were removed and assayed for PCA.

Effect of warfarin on procoagulant activity

Warfarin was administered to normal rats (n = 15) in their drinking water (30 mg/l) until adequate anticoagulation was achieved (Thrombotest clotting times 2-3 times normal values). No efforts were made to avoid coprophagy. The animals were then killed and samples of adrenal, lung, liver and colon removed for procoagulant assay.

Effect of colonic anastomosis

The abdomen was opened through a midline incision under ether anaesthesia. The descending colon was delivered and transected with scissors,