Published in final edited form as:

Platelets. 2016 November ; 27(7): 716–718. doi:10.3109/09537104.2016.1174204.

Platelets are not hyperreactive in patients with ovarian cancer

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Abstract

Paraneoplastic thrombocytosis has been reported in different types of solid tumors, including ovarian epithelial cancer, and found to be associated with a worse outcome. Although the effect of cancer on increasing platelet counts is well documented, the effect of cancer on platelet functions is not well-known. We compared *in vitro* aggregation response of platelets isolated from 34 patients with ovarian cancer to those of platelets from 19 patients with benign ovarian tumors. Aggregation studies were conducted in a light transmission aggregometer, using both a high and a low dose of ADP and collagen. We evaluated platelet preactivation by measuring the plasma concentration of β -thromboglobulin (β -TG) and platelet factor-4 (PF-4) as markers of platelet α granule secretion, using ELISA. We found that ovarian cancer is not associated with an enhanced aggregation response of platelets to ADP or collagen, and plasma concentration of β -TG and PF-4 is not higher in patients with ovarian cancer compared to those in patients with benign ovarian tumors.

Keywords

platelet; ovarian cancer; platelet aggregation; β -thromboglobulin; platelet factor-4

Introduction

Thrombocytosis is present in many patients with solid tumors, including one third of patients with ovarian cancer (1). Despite a general agreement on the presence of a link between

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Author Contributions

S. Feng performed the experiments and analyzed the data; M.H. Kroll interpreted the data and edited the manuscript, A.M. Nick designed the experiments and provided vital reagents; A.K. Sood designed the experiment, interpreted the data, and edited the manuscript, V. Afshar-Kharghan designed the experiments, interpreted the data, and wrote the manuscript.

Declaration of interest

The authors report no conflicts of interest.

cancer and thrombocytosis, platelet function in cancer patients has not been studied adequately. Few reports on platelet function in patients with solid tumors showed differing results from normal to hyperreactive platelets (2;3). Hyperreactive platelets have a lower threshold for aggregation, and hence there may be a higher number of degranulated platelets in circulation and a higher concentration of platelet granular contents in plasma. Because of the clinically significant role that hyperreactive platelets might play in the pathogenesis of thrombotic complications of cancer, we embarked on this study to compare platelet function in 34 patients with ovarian cancer to those in 19 patients with benign ovarian tumors.

Material and Methods

All studies were approved by the Institutional Review Boards of the University of Texas, M.D. Anderson Cancer Center.

Reagents

For platelet aggregation studies, ADP (adenosine-5'-diphosphate), Collagen (from calf skin, Type I), and siliconized micro test tubes were purchased from Bio/Data Corporation (Horsham, PA). ELISA kits for human β -thromboglobulin (β -TG) and human platelet factor-4 (PF-4) were purchased from Novatein Biosciences (Woburn, MA) and Sigma (Saint Louis, MO), respectively.

Collection and processing of blood samples

Whole blood was collected using a 4 × 6 ml vacutainer into a blue-top (sodium citrate) tube with a v:v blood to citrate ratio of 9:1. Blood samples were maintained at room temperature and transferred to the laboratory within two hours. Platelet rich plasma (PRP) was prepared by 15 min of 850 rpm centrifugation at room temperature. Platelet poor plasma (PPP) was prepared by centrifuging PRP samples at 2500 rpm for 20 min at room temperature. All PPP samples were stored at -80°C and the quantity of β -TG and PF-4 was determined by ELISA.

Platelet Aggregation studies

Platelet aggregation studies were conducted in a light transmission aggregometer (8-channel, PC-controlled PAP-8E aggregometer, Bio/Data Corporation). 50 μ l of ADP (at a final concentration of 2 μ M or 20 μ M) and collagen [at a final concentration 19 μ g/ml (0.019 mg/ml) or 190 μ g/ml (0.19 mg/ml)] was added to 450 μ l of PRP. For blank controls, PPP samples were used.

ELISA Assays

To measure β -TG and PF-4, PPP samples were used in the ELISA assays according to the manufacturer's protocol. The optical density (OD) of samples at 450 nm was quantified using a microtiter plate reader (BioTek, Synergy 2) and Gen5 1.05 software.

Results and Discussion

We compared ADP- and collagen-induced platelet aggregation in patients with ovarian epithelial cancer and patients with benign ovarian tumors. To reduce the effect of confounding factors such as surgery or chemotherapy, all blood samples were collected prior to any surgical interventions or chemotherapy. To evaluate platelet aggregation responsiveness, we used both low and high doses of platelet agonists. Because baseline activation status of circulating platelets affects their responses to platelet agonists *in vitro*, we evaluated platelet preactivation by measuring the plasma concentration of β -TG and PF-4 as markers of platelet α granule secretion (4). We found that platelets isolated from ovarian cancer patients showed aggregation responses similar to platelets from patients with benign ovarian tumors (Figure 1A). There was a small but statistically significant difference in the high-dose collagen-induced platelet aggregation between cancer and benign tumor samples, with increased aggregation of platelets from patients with benign tumors ($79 \pm 1.2\%$) in comparison to aggregation of platelets from cancer platelets ($69 \pm 2.6\%$) ($p=0.01$). To investigate *in vivo* preactivation of platelets in cancer patients, we measured β -TG and PF-4 in PPP. We did not detect a higher concentration of β -TG and PF-4 in cancer PPP samples (Figure 1B). In fact, PPP from cancer patients had a lower concentration of both α granule constituents. In the case of PF-4, the difference was statistically significant (9.8 ± 1.7 ng/ml for cancer patients versus 11.7 ± 1.5 ng/ml for patients with non-malignant tumors) ($P=0.01$). These results are different than the two previous reports showing elevated β -TG in lung and breast cancer patients (5;6).

Our negative results raise the hypothesis that different cancers have different effects on platelets, and that platelet activation may not be the only factor involved in the pathogenesis of thrombotic events in ovarian cancer patients. Links between ovarian cancer, thrombosis and platelets may be absent or may involve non-hemostatic platelet functions. However, our study was focused on patients with ovarian cancer prior to any therapeutic interventions, and we cannot rule out possible effects of chemotherapy and surgery on functions and activation of platelets in these patients.

Acknowledgments

This work was supported in part by R01CA177909 (to V.A-K. and A.K.S.), Ovarian Cancer Research Fund (Grant number 258813 to V.A-K. and A.K.S., and Blanton-Davis Ovarian Cancer Research Program (to A.K.S).

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Figure 1. Ovarian cancer and platelet activation

(A) The results of aggregation studies using low and high doses of ADP and collagen on PRP isolated from blood samples of patients with benign ovarian tumor and ovarian cancer are shown as bar graphs representing means $\pm$ standard errors. (B) The concentration of β -TG and PF-4 in plasma samples of patients with benign ovarian tumors as compared to those on patients with ovarian cancer.