Targeting platelet function to improve drug delivery

Mélanie Demers and Denisa D. Wagner*

Immune Disease Institute; Program in Cellular and Molecular Medicine; Children’s Hospital Boston; Department of Pediatrics; Harvard Medical School; Boston, MA USA

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Thrombocytopenia-induced tumor hemorrhage improves drug delivery to tumors. This phenomenon presents a new way to increase drug efficacy with minimal side effects. Combining anti-platelet treatment with therapeutic drugs may help us in the search for more effective ways to fight cancer.

We recently showed that platelet depletion increased the efficacy of chemotherapy and reduced tumor growth in mice. Platelets are the cellular orchestrators of primary hemostasis. They have long been known to prevent bleeding upon injury by their ability to induce coagulation and thrombus formation. Through expression of adhesion molecules and release of their granule contents, they modulate the immune system and preserve vascular integrity. Platelets also have a protective role during the inflammatory response. Our group has shown that, in thrombocytopenic animals, induction of local inflammation results in severe bleeding originating from capillaries and post-capillary venules at the inflamed site. The observed hemorrhage correlates with the presence of inflammatory cells suggesting that platelets protect blood vessels from injurious leukocytes.

In cancer, platelets have been known for their potential to promote metastasis. They have been shown to support angiogenesis, and promote cancer cell survival and adherence to the endothelium, all of which contributes to the spreading of cancer to distant sites. In the primary tumor, platelets may modulate the growth of tumor vessels by differential release of pro- and anti-angiogenic factors. Recently, we found a new role for platelets in cancer: the maintenance of tumor vessel integrity. We showed that injection of platelet-depleting antibody in tumor-bearing mice induced rapid bleeding in and surrounding the tumor without affecting vessels elsewhere in the body. Since only tumor vessels were affected by the low platelet count, we hypothesized that opening of the tumor vasculature would promote the delivery of circulating drugs to the tumor. We thus combined platelet depletion with the chemotherapeutic agent paclitaxel and showed a significant reduction in tumor growth compared with paclitaxel alone. This combined treatment increased the accumulation of the drug at the tumor site thereby increasing its efficacy, as observed by increased tumor cell apoptosis and reduced proliferation, without increasing toxic side-effects to other organs. Thus, thrombocytopenia increased the efficacy of the chemotherapeutic treatment by causing a higher quantity of drug to be delivered specifically to the tumor site.

Using low platelet counts to improve drug delivery to tumors provides a new way to increase drug access and efficacy that could be applied to a variety of tumors. Tumor hemorrhage was observed upon platelet depletion in several different tumor types, such as subcutaneous lung carcinoma, mammary carcinoma and melanoma as well as established lung metastasis in mice. Moreover, similar to the localized bleeding upon inflammatory challenge, the tumor hemorrhage associated with platelet depletion occurred at sites of neutrophil and macrophage accumulation. Induction of thrombocytopenia in mice deficient in leukocyte adhesion molecules β2 and β3 integrins, which are characterized by a reduction in infiltrating neutrophils and macrophages in the tumor stroma, results in a significant reduction in tumor hemorrhage. This strongly suggests that, in the tumor, the inflammatory cells are responsible for the bleeding associated with low platelet counts (Fig. 1A and B). Because platelets prevent tumor vessel injuries caused by infiltrating leukocytes, and most tumors contain inflammatory leukocytes, lowering platelet count to improve drug delivery should be feasible in a large variety of tumors.

In addition, low platelet counts could be used for the delivery of a wide range of therapeutic drugs (Fig. 1C). The drugs evaluated in our study are not tumor specific and thus accumulate in the tumor through passive permeability. Platelet depletion induces breaches in the tumor vasculature enabling more drug to cross the endothelium. In addition to accumulation of a chemotherapeutic drug, we showed that fluorescently-labeled 1 µM microspheres infused i.v. were accumulating in the hemorrhagic tumor. This suggests that the delivery of drug-containing nanoparticles allowing a slow release of the drug can also be improved by pairing with thrombocytopenia. The use of...
antigen-specific therapy, such as monoclonal antibodies and antibody-enzyme fusion proteins, may have an even bigger effect on tumor growth when paired with platelet depletion. The combination of direct specificity for the tumor with a better access to the target would certainly be beneficial. Even the combination of low platelet counts with the newest immunotherapeutic strategies, such as dendritic cell vaccines and adoptive T cell transfer, would be applicable. It will be important to identify the factor(s) released by platelets that constitutively prevent tumor hemorrhage and learn how they could be inhibited. Employing specific inhibitors of platelet function or neutralizing agents would enable the development of new treatments without the need for lowering platelet count. Such anti-platelet treatment combined with chemotherapy or immunotherapy would enhance their delivery to the tumor and may represent a significant improvement in our ability to fight cancer with minimal side effects and without increasing the risk for bleeding.

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**Disclosure of Potential Conflicts of Interest**
The authors declare that they have no conflict of interest and no competing financial interests.
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