Analysis of Prothrombotic Mechanisms and Endothelial Perturbation during Treatment with Angiogenesis Inhibitors

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Our department participated in multi-center phase II trials investigating the efficacy and toxicity of the angiogenesis inhibitor SU5416 in patients with advanced renal cell carcinoma, melanoma, or soft tissue sarcoma [1]. SU5416 is a small synthetic molecule that targets the tyrosine kinase domain of the vascular endothelial growth factor receptor (VEGFR)-1 and -2, thereby preventing subsequent intracellular signaling [2–4]. SU5416 furthermore inhibits c-kit [5]. Three of the 17 patients entered at our department developed a thromboembolic event. Finally, 5 in total of 80 (6.3%) patients entered developed a thromboembolic event, which is a normal incidence of a patient population with cancer. We investigated whether SU5416 affected the coagulation cascade and/or endothelial cells [6]. We determined the coagulation parameters thrombin-antithrombin (TAT) complexes, prothrombin activation fragments 1+2 (F1+2), endogenous thrombin potential (ETP), activated protein C (APC) resistance, thrombin-activatable fibrinolysis inhibitor (TAFI) antigen, soluble thrombomodulin (s-TM), and the fibrinolytic parameters, tissue-plasminogen activator antigen (t-PA) and plasminogen activator inhibitor-1 antigen (PAI-1). The endothelial cell (EC) parameters von Willebrand factor (vWF), soluble(s)-E-selectin, and soluble-tissue factor (s-TF) were measured. We observed no change in TAT complexes, F1+2 levels, APC resistance, TAFI, s-TM and fibrinolytic parameters. A significant

increase in ETP levels was observed in all patients. Moreover, the ETP increased to a significantly greater extent in the three patients experiencing an event compared to the 14 patients without an event. We observed furthermore significant increases of the EC parameters, especially s-E-selectin and s-TF, which increased to a significantly greater extent in the patients experiencing an event. Interestingly, mean basal levels of the EC parameters were significantly higher in patients experiencing an event compared to those without an event. In conclusion, endothelial cell perturbation occurred during treatment with SU5416 and probably resulted in an increased risk of thromboembolic events.

That SU5416 increases the risk on thromboembolic events was confirmed by the fact that 9 thromboembolic events occurred in 8 out of 19 patients during a phase I trial investigating the feasibility of cisplatin-gemcitabine combined with SU5416 [7]. We had the opportunity to obtain blood samples to analyze coagulation (TAT, F1+2, ETP) and EC (vWF, s-E-selectin, s-TF) parameters in three patients, two of whom developed a thromboembolic event [8]. The results were compared with measurements in 6 patients treated with cisplatin-gemcitabine alone, and the 17 patients treated with SU5416 alone. During the first and second cycles of cisplatin-gemcitabine plus SU5416, all three patients exhibited similar, clearly cycle-dependent,

increases in both the coagulation and endothelial cell parameters. Despite the fact that SU5416 was infused on day 18, both the coagulation and endothelial cell parameters decreased after day 18 of the first course, returning to basal values by day 1 of the second cycle. Opposite cyclic fluctuations in platelet number were observed. Statistical analysis showed that the change in platelet number had a significant negative predictive effect on s-E-selectin levels. Significant activation of the coagulation cascade only was observed in the patients treated with cisplatin/gemcitabine alone, whereas in patients treated with SU5416 alone, significant endothelial cell activation was observed.

In conclusion, VEGF is, besides a permeability, proliferation, and migration factor, also a maintenance and protection factor for endothelial cells. We furthermore hypothesize that endothelial cells deprived of VEGF after exposure to SU5416, became activated and more susceptible to damage during treatment with cisplatin/gemcitabine, which was aggravated by a transient decrease in platelets, which are, amongst other things, carriers of VEGF. These results suggests that platelets may have trophic effects on endothelial cells and may play a role in maintaining vascular integrity.

References

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