THERAPY

Opposition: biphasic pharmacodynamics

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The αvβ3 and αvβ5 integrins are important for cell–cell adhesion and their expression on endothelial cells facilitates a number of cellular processes that are important for angiogenesis. RGD mimetics (analogues of the Arg–Gly–Asp sequence) that inhibit both of these integrins, such as cilengitide and S 36578, are considered promising anticancer drugs, but clinical testing has not been as successful as was hoped. Andrew Reynolds, Kairbaan Hodivala-Dilke and colleagues now reveal worrying pro-angiogenic effects of these inhibitors.

Reynolds and colleagues treated mice bearing B16F0 melanoma or Lewis lung carcinoma (LLC) grafts, which express αvβ3 integrin, with cilengitide or S 36578 and found that blood plasma concentrations of the inhibitors rapidly fell from micromolar to nanomolar concentrations. As nanomolar concentrations were sustained for 16–24 hours after administration, they investigated whether nanomolar concentrations have differential effects compared with micromolar doses. Using osmotic minipumps, they treated mice bearing B16F0 or LLC grafts with nanomolar concentrations of cilengitide or S 36578 and observed a significant increase in tumour growth, which did not occur after treatment with concentrations of ~4 μM. They also treated mice bearing A375 human melanoma xenografts — which respond to RGD mimetics — with nanomolar concentrations and again found that tumour growth increased. Tumour growth due to nanomolar concentrations was prevented in β3 integrin and β5 integrin double-knockout mice, indicating that the drugs affect host cells rather than directly modulating tumour cells. As tumour endothelial cells are host cells that express αv integrin, the authors reasoned that these were the target, and analyses of LLC and B16F0 tumour sections revealed that nanomolar concentrations promoted vascularization. Using an *ex vivo* aortic ring assay to assess angiogenesis, they observed a biphasic dose response wherein nanomolar doses increased vascular endothelial growth factor A (VEGFA)-stimulated angiogenesis but doses exceeding 20 μM inhibited angiogenesis. Further analyses revealed that cyclical exposure to nanomolar and micromolar plasma concentrations antagonized the anti-angiogenic effects of higher doses.

So, how do these opposing effects occur? Nanomolar concentrations of cilengitide or S 37578 neither promoted endothelial cell adhesion nor activated αvβ3 integrin. However, levels of endothelial VEGF receptor 2 (VEGFR2) protein but not of its mRNA were increased, and blocking VEGFR2 with antibodies suppressed the VEGFA-dependent angiogenic effects of nanomolar concentrations *ex vivo* and *in vivo*. Moreover, after exposure to nanomolar doses, VEGFA-induced degradation of VEGFR2 was attenuated in endothelial cells. VEGFR2 accumulated in recycling vesicles in a RAB4A-dependent manner and VEGFA-mediated endothelial cell migration was increased. In addition, there was increased recycling and delivery of αvβ3 to focal adhesions. These data suggest that exposure to nanomolar doses of RGD mimetics, which is likely to occur in the interval between drug administrations, promotes angiogenesis and hence tumour growth through increased Vegf-induced migration of endothelial cells, mediated by increased recycling of VEGFR2 and αvβ3 integrin.

These data further highlight the need to carefully consider how new drugs, such as anti-angiogenesis drugs, are used. In this case the authors suggest that alternative drug administration procedures that are currently undergoing Phase I clinical testing — such as pump-based systems, which should deliver a constant dose — should be more successful for drugs that exhibit such biphasic pharmacodynamics.

Gemma K. Alderton

ORIGINAL RESEARCH PAPER
Reynolds, A. R., *et al.* Stimulation of tumor growth and angiogenesis by low concentrations of RGD-mimetic integrin inhibitors. *Nature Med.* 22 Mar 2009 (doi: 10.1038/nm.1941)
FURTHER READING Mosesson, Y. *et al.* Derailed endocytosis: an emerging feature of cancer. *Nature Rev. Cancer* **8**, 835–850 (2008)