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A critical role for RhoA-GTPase signaling in the tumor vascular disrupting action of combretastatin-A4-phosphate *in vivo*

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Abstract

Background: Tubulin binding microtubule depolymerising agents form a growing group of tumor vascular disrupting agents (VDAs) in clinical trial, with combretastatin A-4-phosphate (CA4P) the lead compound. Signalling through RhoGTPase/ROCK-dependent pathways is central to CA4P-induced effects on endothelial cells *in vitro* (1). Here, we tested the hypothesis that RhoGTPase/ROCK signalling is also important in tumor vascular damaging effects *in vivo*.

Experimental procedures: SW1222 human colorectal carcinoma cells were grown as solid sub-cutaneous tumors in SCID mice. The Rho kinase (ROCK) inhibitor, Y-27632 (50 mg/kg) or saline vehicle, was administered intraperitoneally (i.p.), 5 minutes prior to 100 mg/kg CA4P or saline i.p. Laser Doppler flowmetry was used to assess tumor vascular response from 0 – 2h post-treatment, under isoflurane anaesthesia. Intravenous administration of fluorescent tomato lectin was used for assessing tumor perfusion at 1, 3, 6 and 24 hours post-CA4P/saline treatment, unanaesthetised. Necrosis (H&E) and leukocyte infiltration (immunohistochemistry) were assessed at 24h from excised tumors. Mean arterial blood pressure (MABP) was monitored in unanaesthetised mice without tumors, using a tail cuff system.

Results: Y-27632 alone significantly decreased MABP by 40% but did not significantly affect tumor necrosis at 24 hours (17±4% of tumor sectional area for treated versus 10±3% for controls). Y-27632 alone reduced relative red cell flux in the tumor (laser Doppler flowmetry) by approximately 50%, which was similar to the reduction observed for CA4P alone. However, pre-treatment with Y-27632 did not affect CA4P-induced laser Doppler or perfused vascular volume measurements in the first few hours after CA4P and significantly reduced the effect of CA4P on perfused vascular volume and necrosis measured at 6 and 24 hours. Necrosis was 61±5% for CA4P alone and 35±7% for Y-27632+CA4P. These changes were accompanied by a decrease in staining for the myeloid markers, myeloperoxidase and GR-1 with Y-27632 pre-treatment.

Conclusions: The Y-27632-induced decrease in MABP is consistent with the decrease in relative red cell flux in the tumor, via a decrease in tumor perfusion pressure. Despite these effects of Y-27632 alone, its administration prior to CA4P was protective of CA4P-induced vascular damage at the later time-points, suggesting that ROCK inhibition is acting downstream from initial vascular shut-down, potentially via modulation of myeloid cell recruitment. These data indicate that RhoGTPase/ROCK-dependent signalling is a critical factor in determining extent of vascular disruption by CA4P – these results have significance for similar VDAs in development.

References

(1) Kanthou C. & Tozer GM, 2002, Blood 99: 2060-2069.

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