Signaling interactions between RhoGTPase and cAMP/cGMP influence endothelial responses to the vascular disrupting agent combretastatin A4 phosphate

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Background

Combretastatin A4 phosphate (CA4P) is a tumour vascular disrupting agent (VDA) that targets endothelial microtubules, triggering remodelling of the actin cytoskeleton, contractility and disruption of VE-cadherin junctions through RhoGTPase/ROCK-dependent pathways. These events lead to a rise in endothelial monolayer permeability. A rise in permeability is considered crucial for vascular shutdown elicited by CA4P *in vivo*. CA4P also inhibits endothelial migration and induces mitotic arrest and apoptosis, so potentially it could also target tumour angiogenesis.

Method

In this study, the nature of signalling interactions between Rho/ROCK and cAMP/cGMP and their influence on cytoskeletal and functional responses of endothelial cells to CA4P were investigated.

Results

Several cAMP/cGMP analogues inhibited CA4P-induced Rho/ROCK activation and prevented actin remodeling, disruption of cell-to-cell junctions and permeability rise in endothelial monolayers. cAMP inhibits Rho by either protein kinase A (PKA)-dependent mechanisms or via activation of Epac1/Rap1. *O*-Me-cAMP, an analogue that selectively activates Epac1/Rap1 abolished activation of Rho/ROCK by CA4P while selective PKA activator 6-Bnz-cAMP only partially inhibited Rho/ROCK activation and actin remodelling by CA4P. Inhibitors of PKA did not alter endothelial responses to CA4P in the presence of cAMP analogues suggesting that cAMP acts primarily via Epac1/Rap1 to inhibit Rho/CA4P interactions. CA-4-P also inhibited endothelial migration and abolished lamellipodia at the leading edge of migrating cells in injured monolayers. Rho inhibitor C3 exoenzyme and ROCK inhibitor Y27632 as well as cAMP analogues re-established cell movement and formation of lamellipodia in wounded monolayers exposed to CA-4-P, suggesting that inhibitory effects on migration were mediated via Rho/ROCK.

Conclusion

Deciphering molecular pathways that modulate endothelial responses to VDAs is important for further targeting. Our data demonstrate that interactions between cGMP/cAMP and Rho influence both the

vascular disrupting and anti-angiogenic activities of CA-4-P and point to cAMP/cGMP as potential targets for improving VDA activity.

References:

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