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A Pharmacological Profile of Receptor Tyrosine Kinase Inhibitors on VEGFR2-Stimulated NFAT Signalling in HEK-293 Cells.

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Anti-vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitors (RTKIs), such as cediranib, are currently used in the clinic as adjuvant anti-angiogenic treatments in a variety of solid tumours (1). However, their pharmacological characteristics in a whole cell system have not been extensively explored. Here, we have investigated the characteristics of four RTKIs (cediranib, sorafenib, pazopanib and vandetanib) on VEGF-stimulated NFAT signalling in HEK-293 cells expressing the human VEGF receptor 2 (VEGFR2).

HEK-293 cells expressing the human VEGFR2 and an NFAT luciferase reporter gene (Promega) were cultured in DMEM +10% FCS at 37^{o}C in 5% CO_2 to confluence before being seeded in white walled 96 well plates at 4x10^{4} cells/80µl in DMEM +0.1%BSA (medium). Cells were treated with RTKIs (30µM–100pM; added in 10µl medium) for 1h (37^{o}C, 5% CO_2) prior to addition of $VEGF_{165}$ (100nM–30pM; added in 10µl medium) for an additional 5h (37^{o}C in 5% CO_2). Luciferase activity was measured using the One-Glo® Luciferase Assay System (Promega), according to manufactures instructions. IC_{50}, EC_{50} and E_{max} values were calculated using GraphPad Prism v6.0. Values are mean $\pm$ SEM of n replicate experiments. In each individual experiment, 4 replicates were made for each condition.

VEGF caused a concentration dependent increase in the expression of the NFAT reporter gene (log EC_{50} =9.57±0.02, maximum fold over basal = 10.7±7.06; n=5). The response to 1nM $VEGF_{165}$ was inhibited by vandetanib (log IC_{50} = -6.72±0.03; n=5), pazopanib (log IC_{50}= -8.25±0.03; n=5), cediranib (log IC_{50}= -9.13±0.01; n=5) and sorafenib (log IC_{50}= -8.02±0.06; n=5). All RTKIs were shown to mediate a non-competitive antagonism of the VEGFR2 response (Table 1).

	$VEGF_{165}$		$VEGF_{165}$ + RTKI	
	pEC_{50}	% E_{max} (normalised to 10nM $VEGF_{165}$ response)	pEC_{50}	% E_{max} (normalised to 10nM $VEGF_{165}$ response)
cediranib(3nM)	9.68±0.09	100	9.13±0.18	10.97±2.27
pazopanib(10nM)	9.66±0.11	100	9.14±0.16	32.84±1.99
sorafenib(30nM)	9.72±0.06	100	8.89±0.09	18.91±8.93

vandetanib (300nM)	9.90±0.14	100	9.41±0.14	29.47±7.85

Table 1. Effect of RTKIs on VEGF concentration-response parameters.

Increasing concentrations of each RTKI lead to a progressive decrease in E_{max}. The small shift in the VEGF pEC_{50} was significant for all RTKIs ($p<0.05$) (two-way ANOVA). Table 1 shows the effect of each RTKI used at the highest concentration. Cediranib n=6, pazopanib n= 5, sorafenib n=7, vandetanib n=5.

These data show that the VEGF-mediated NFAT reporter system provides a robust and quantitative assay to study the impact of RTKI inhibitors on VEGFR2 signalling in intact cells. The rank order of potency obtained for these four RTKIs in intact cells (cediranib>pazopanib>sorafenib>vandetanib) agree with previous reports obtained in purified VEGFR2 catalytic domain fragments (2).

1. Bagri A et al. (2010). Trends Mol Med **16**: 122-132.

2. Davis MI et al. (2011). Nature Biotechnol **11**: 1046-1052.