The AKT Kinase Inhibitor GSK690693 Activates the Mast-Cell Degranulation Receptor, Mrgprx2, and Induces Adverse Histamine-Like Reactions in Dogs

Mrgprx2 is a family-A G-protein-coupled receptor expressed on human mast cells. Mrgprx2 and its orthologous receptors in other mammalian species are relatively promiscuous, being activated by structurally diverse peptides and small molecules (basic secretogogues). Activation of Mrgprx2 leads to mast cell degranulation and histamine release, and underlies acute histamine-like adverse drug reactions to various injected therapeutic agents. An example is the bradykinin antagonist peptide icatibant, which activates Mrgprx2 and causes injection site reactions (ref.1). We investigated whether activation of Mrgprx2 might be responsible for unexplained histamine-like adverse reactions occasionally observed for drug candidates in nonclinical stages of development. To measure Mrgprx2 agonist activity, Chinese hamster ovary (CHO-K1) cells stably expressing a tagged version of the receptor were used. Known Mrgprx2 agonists (Compound 48/80, cortistatin-14 and substance P) stimulated concentration-dependent intracellular calcium release and β-arrestin association (Table 1), consistent with published data (refs.1&2). The pan-AKT kinase inhibitor GSK690693 (ref.3) caused overt histamine-like reactions upon intravenous infusion into dog at doses of 25mg/kg and above, which produced exposures (C_{max}) ≥6μM (total drug). Symptoms were attenuated by administration of antihistamines and steroids. Infusion of GSK690693 to rats and monkeys (C_{max} ≥10μM) also resulted in histamine-like clinical signs (e.g. emesis), though elevated plasma glucose resulting from AKT inhibition may have been a confounding factor. In vitro, GSK690693 caused intracellular calcium release in the Mrgprx2-CHOK1 cells (Table 1). GSK690693-evoked calcium release was also observed in human U2OS cells transiently transfected with an untagged version of Mrgprx2, but not in Mrgprx1-expressing or host U2OS cells. GSK690693 showed low to moderate plasma protein binding (55%, 56%, 59% and 70% in rat, dog, monkey and human, respectively). In conclusion, Mrgprx2 agonism of GSK690693 was the likely mechanism for the in vivo histamine-like adverse effects observed in dogs. These findings support the need for further work to validate Mrgprx2 activity as a screening assay for predicting histamine-like reactions in vivo.

Table 1: Agonist activity in Mrgprx2-CHOK1 cells (pEC_{50},mean±SD)

	Calcium release	**β-arrestin**
Compound 48/80	6.55 (n=2)	5.89 (n=1)
Cortistatin-14	6.9±0.31 (n=3)	7.1±0.16 (n=2)
Substance P	6.49 (n=1)	6.45 (n=1)
GSK690693	5.11 (n=1)	ND

Refs:

(1) McNeil et al. *Nature* (2014) **519**, 237–241.
(2) Southern et al. *J Biomol Screen* (2013) 18(5), 599–609.
(3) Rhodes et al. Cancer Res (2008) 68(7), 2366–2374.