Utility Of A Knock-Out Mouse Model In Determining The Mechanism Of Action Of A GPCR agonist

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Several agonists with a high level of in-vitro potency against a class A GPCR thought to be involved in glycaemic control have been shown to lower blood glucose area under the curve (AUC) in a mouse oral glucose tolerance test (OGTT) model. To determine if these glucose lowering effects were being conferred via the intended mechanism of action a receptor knock-out (KO) mouse model was generated. The same compounds were then assessed in an OGTT run in both receptor KO mice and wild-type (WT) control littermates.

In each study compound dose was set with the aim of reaching free levels of systemic exposure several times above the in-vitro mouse receptor potency. Assessment of historical OGTT data ensured that each experiment used group sizes that allowed the studies to be correctly powered to determine if a statistical difference was present between vehicle and compound treated groups. Vehicle (0.1% pluronic F127) or a compound suspension was administered po to both WT and KO animals 30 minutes prior to an oral glucose load of 2g/kg (10ml/kg) in water. Blood glucose levels were measured from a tail prick blood sample using an Aviva AccuChek glucose monitor prior to compound administration and then at 0, 10, 25, 40, 60 and 90 minutes post glucose load. Cardiac puncture blood samples were taken under terminal anaesthesia for compound exposure level determination. Blood glucose AUC was calculated from time 0 baseline glucose levels for each group and statistical significance assessed using ANCOVA analysis (significance p<0.05).

Compound A significantly reduced mean blood glucose AUC (p<0.001) in both WT and KO animals by 50.4% (9.08±0.42 vs. 4.51±0.30mmol/l*h) and 43.7% (9.17±0.39 vs. 4.95±0.45mmol/l*h) respectively compared to vehicle. No difference was found between WT and KO response indicating compound effects were generated by an off-target mechanism. Compound B reduced mean blood glucose AUC (p<0.001) by 45.0% in WT (8.60±0.41 vs. 4.72±0.41mmol/l*h) and 32.0% in KO 8.98±0.59 vs. 6.10±0.59mmol/l*h). However, inhibition of glucose excursion profile in KO animals was found to be significantly less (p<0.01) than that in WT animals suggesting this was a combination of both on and off target effects. Compound C reduced blood glucose AUC by 29.5% in WT animals (8.60±0.41 vs. 6.06±0.47mmol/l*h, p<0.001) but had no effect in KO (8.98±0.59 vs. 9.35±0.68mmol/l*h) indicating that this compound had the desired mechanism of action.

These data show that in-vitro potency alone cannot be used as a guide to how a compound is mediating its in-vivo effects. Use of receptor KO mice enabled determination of which compounds were lowering blood glucose via the desired GPCR of interest. This enabled an early stop decision on a flawed series of compounds and for work to be transferred to a series proven to act by the desired mechanism of action.