INTRODUCTION

The binding of compounds to their target receptor is critical for achieving efficacy. The potential advantages of influencing binding kinetics of ligands to their receptor include:

- clinical efficacy,
- duration of action,
- safety margins and
- compound differentiation amongst others.

Both literature and in-house in vitro functional binding data suggested that our series of non-peptide CRF₁ antagonists displayed insurmountable antagonism.

i) Could this be due to slow off set kinetics?
ii) could slow off-set kinetics be used to improve duration of efficacy?

OBJECTIVES

Optimise the use and analysis of data from a non-equilibrium binding assay to measure the kinetics of these compounds. Use the kinetically derived association and dissociation rates in conjunction with the compounds pharmacokinetic parameters in the rat to simulate the receptor occupancy vs. time profiles. These simulations would be used as a replacement for in vivo receptor occupancy studies to enable:

- faster triage of compounds with slow offset at an early stage of discovery
- quicker progression to compound selection and first in man.

This approach is based on the principle that compounds with slower off-set from the receptor have the potential to sustain the duration of efficacy due to an increased residence time at the receptor.

METHODS

Data from the non-equilibrium binding assays was fitted in NONMEM v6.2 using a single competitive binding model to obtain estimates of the compounds association and dissociation rates. Receptor occupancy vs. time data was modelled in NONMEM v6.2 and further simulations were performed in Berkley Madonnan.

RESULTS – IN VITRO

Varying degrees of competitive and non-competitive antagonism was observed in the functional assay (Fig 1).

In these experiments the assumption is that equilibrium has been reached, however this will not be the case for compounds with slow off-set kinetics, where the dissociation half-life is much longer than the experiment time-frame. Therefore the observed potency from these experiments may not accurately reflect the compounds ‘true’ potency.

There appeared to be a strong correlation between the compounds Kᵦᵣᵣᵦ rate and it’s ability to suppress the tracer compound in the functional assay, supporting the slow off-set theory (Fig 3).

There were clear discrepancies between the compounds kinetic and in vivo derived EC50 values (Table 2).


table 2: Kinetically derived parameters from PKPD modelling of in vitro non-equilibrium binding kinetic data, and in vivo derived EC50


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RESULTS – IN VIVO

Simulations of the occupancy vs. time profiles using the in-vivo derived parameters (Fig 6, solid line), showed a poor representation of the in vivo data. Using Berkley Madonnana it was possible to optimise the kinetic parameters to the in vivo data (Fig 6, dashed line). These optimised parameters predicted Kᵦᵣᵦ in line with those determined from the E₀ᵦᵣᵦ PKPD model. However, the Kᵦᵣᵦ was different for most compounds from the in-vivo predicted value (Table 3).

CONCLUSIONS

CRF₁ antagonists displayed a range of off-set kinetics. There appeared to be a consistent discrepancy between the in vitro and in vivo receptor association rates, however the rank order of the compounds in terms of their rate of dissociation from the CRF₁ receptor translated well.

Therefore, this PKPD model based approach proved to be useful to triage between compounds at an early stage of the project where it is not feasible to perform in vivo receptor occupancy studies on a large number of compounds. The in vivo study would still be required at candidate selection to confirm the simulated profile.

REFERENCES

2) Renger et al, 2006 Br J Pharmacol 149(7): 941-947
4) Benson et al, 2010 Br J Pharm 156(2): 389-398