Model based analysis of antagonist binding kinetics at CRF_1 receptors *in vitro* and *in vivo*

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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[1].

Both literature[2] and in-house *in vitro* functional binding data suggested that our series of non-peptide CRF_1 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[3].

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.[4] Receptor occupancy vs. time data was modelled in NONMEM v6.2 and further simulations were performed in Berkley Madonna.

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.

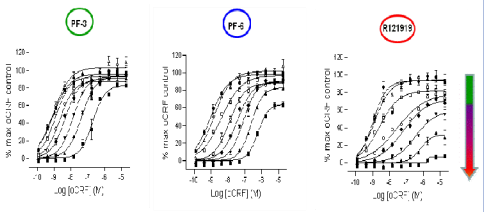

Fig 1: Effects of multiple concentrations of CRF_1 antagonists in the functional assay

To solve the issue of assay time to reach equilibrium, a non-equilibrium binding kinetic assay was used, which was an optimised version of the method first described by Mahan and Motulsky[3]. The data was simultaneously fitted in NONMEM v6.2 to calculate k_{on} and k_{off} rates from which K_d (k_{off}/k_{on}) and dissociation half-life values could be calculated[4] (Fig 2,Table 2).

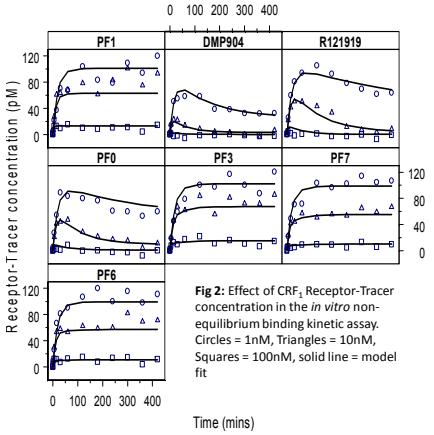

Fig 2: Effect of CRF_1 Receptor-Tracer concentration in the *in vitro* non-equilibrium binding kinetic assay. Circles = 1nM, Triangles = 10nM, Squares = 100nM, solid line = model fit

Compounds ranked in same order, and affinities similar for less potent molecules. However, large difference in estimated affinity for the compounds with slow off-set kinetics (Table 1).

Compound	Kd (pM)	Ki (pM)
Tracer	5000	8000
PF-7	8180	8000
PF-3	13900	14700
PF-1	12400	10600
PF-6	3670	4000
DMP-904	279	1900
R-121919	303	3800

Table 1: Comparison between potency estimates. Kd from kinetic assay, Ki from functional assay.

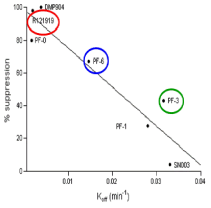

Figure 3: Correlation between the off rate (min^{-1}) of each antagonist from the rat CRF_1 receptor and percentage suppression of a 20μM oCRF response by 20μM of each of the CRF_1 antagonists in the cAMP functional assay.

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

Compound	*In vitro* Kinetic Parameters: Kd (nM)	k_{on} ($M^{-1}min^{-1}$)	k_{off} (min^{-1})	Dissociation Half-life (hr)	*In vivo* EC50 (nM)
SN003 (tracer)	3.64 ±0.59	$7.72e^6$ ±1.6e^6	0.026 ±0.004	0.44	ND
SN003	5.62 ±0.53	$6.2e^6$ ±8.9e^5	0.033 ±0.004	0.35	ND
PF-3	8.22 ±1.48	$4.06e^6$ ±4.6e^5	0.0316 ±0.004	0.37	ND
PF-1	4.24 ±1.2	$7.88e^6$ ±1.8e^6	0.028 ±0.008	0.41	ND
PF-6	3.06 ±0.39	$4.6e^6$ ±5.1e^5	0.0146 ±0.003	0.79	13.4 (0.002)
DMP-904	0.275 ±0.07	$1.47e^7$ ±1.4e^6	0.0038 ±0.001	3.04	ND
R121919	0.549 ±0.15	$3.69e^6$ ±6.1e^5	0.0019 ±0.0005	6.08	0.290 (0.0003)
PF-0	0.734 ±0.17	$2.79e^6$ ±6.4e^5	0.0017 ±0.0002	6.79	1.48 (0.002)

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

RESULTS – *IN VIVO*

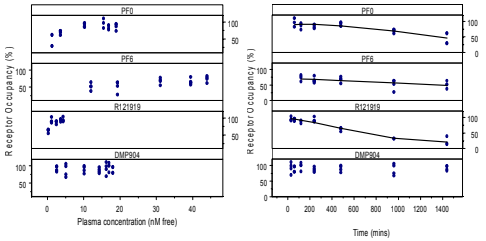

Fig 4: Concentration vs. Receptor Occupancy (left hand panel), and Receptor Occupancy vs. Time (right hand panel) for four CRF_1 antagonists, solid line = model fit to the data.

Despite slow off set kinetics none of the compounds displayed hysteresis when looking at concentration vs. occupancy plot. (Fig 4) Therefore a direct Emax model could be fitted to the data using NONMEM to estimate an *in vivo* EC50 for all compounds except DMP-904 where the occupancy was maximal for the whole experiment (Fig 4,Table 2).

Hysteresis was not observed for these compounds due to a non-optimal study design, as the first time-point (30 min – 2h) missed the compound's absorption phase.

Simulations for R121919 using the *in vitro* derived kinetic parameters (Fig 5) shows that had samples been taken earlier then hysteresis would have been observed.

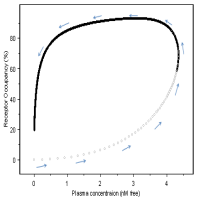

Fig 5: Simulation of R121919 receptor occupancy vs. concentration profile using *in vitro* kinetic parameters. Arrows indicate time order, and black line represents concentration range covered in the *in vivo* study.

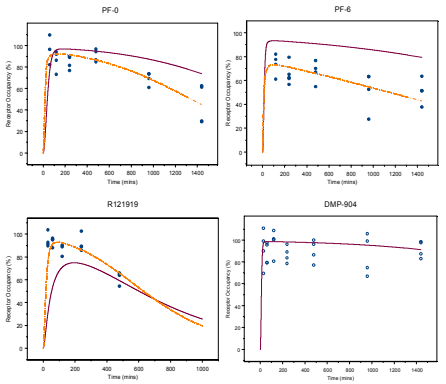

Fig 6: Simulations of Receptor Occupancy vs. Time profiles, using *in vitro* parameters (solid line) and *in vivo* optimised parameters (dashed line), observed receptor occupancy data (circles).

Simulations of the occupancy vs. time profiles using the *in vitro* derived parameters (Fig 6, solid line), showed a poor representation of the *in vivo* data. Using Berkeley Madonna it was possible to optimise the kinetic parameters to the *in vivo* data (Fig 6, dashed line). These optimised parameters predicted K_d in line with those determined from the E_{max} PKPD model. However, the k_{off} rate was different for most compounds from the *in vitro* predicted value (Table 3).

Compound	*In vitro* k_{off} (min^{-1})	*In vivo* k_{off} (min^{-1})
PF-0	0.0017	0.0238
PF-6	0.0146	0.237
R121919	0.0019	0.0034
DMP904	0.0038	ND

Table 3: Comparison of the *in vitro* and *in vivo* dissociation rate constants. ND=Not determined

CONCLUSIONS

CRF_1 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 CRF_1 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

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