PHARMACOKINETIC/PHARMACODYNAMIC PROPERTIES OF SMALL MOLECULE CXCR3 ANTAGONISTS

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Pharmacokinetic/pharmacodynamic (PK/PD) models are essential to the drug discovery process (Chien et al., 2005) and enable discrimination between compounds and dosing regimens and the nature of the PK/PD relationship. Here we describe the use of a murine CXCR3 internalisation assay (Jopling et al., 2006) and its utility for analysing the PK/PD relationship of the small molecule CXCR3 antagonist, NBI-74330 (Heise et al., 2005).

All work was carried out in accordance with the Animals (Scientific Procedures) Act 1986. Splenocytes from 8-week-old DO11.10 mice were cultured with ovalbumin peptide (OVA_{323-339}) (200ng.ml^{-1}) and murine IL-2 (10ng.ml^{-1}) for 8 days in vitro. Activated DO11.10 cells (5x10^5) were resuspended in EDTA-treated pooled plasma and incubated, with agitation, for 60 minutes at 37°C with agonist ± antagonist as appropriate. Cells were then washed and incubated with anti-murine CXCR3 antibody for 30 minutes on ice. Unbound antibody was removed by washing and samples were fixed and acquired by flow cytometry. For PK/PD analysis, mice were dosed with NBI-74330 (100mg.kg^{-1}) orally (p.o.) or subcutaneously (s.c.) and plasma samples taken at specified timepoints. Samples were extracted by protein precipitation and the resulting extracts analysed by LC-MS/MS. Apparent pA2 values were estimated from significant rightward shift of the CXCL11 E/[A] curve in the presence of antagonist.

NBI-74330 plasma exposure was greater following p.o. compared to s.c. administration, as indicated by C_{max} (p.o. 7051-13010ng.ml^{-1}; s.c. 1047-4737ng.ml^{-1}) and AUC (p.o. 21603-41349ng.h.ml^{-1}; s.c. 5702-21600ng.h.ml^{-1}). However dosing of NBI-74330 generated an N-oxide metabolite (metabolite 1) with 4-fold higher affinity value in the plasma CXCR3 receptor internalisation assay (pA2 values: NBI-74330, 6.42±0.07; metabolite 1, 7.04±0.16). Plasma C_{max} for metabolite 1 was higher following p.o. compared to s.c. administration (p.o. 4846-6276ng.ml^{-1}; s.c. 1005-2842ng.ml^{-1}) whilst AUC was similar between dosing regimes (p.o. 8149-14495ng.h.ml^{-1}; s.c. 8897-17704ng.h.ml^{-1}). Overall, the concentrations of both NBI-74330 and metabolite 1 were higher at 24h following s.c. administration, demonstrating that sustained exposure was achieved via this regimen. The corresponding PD data indicated >30-fold antagonism of the CXCL11 E/[A] curve at 1h and 4h (p<0.01) but not at 24h post p.o. administration, whereas s.c. administration resulted in >30-fold antagonism of the CXCL11 E/[A] curve at 2h and 24h post dose (p<0.001). Analysis of the oral PK/PD relationship showed no evidence of hysteresis, indicating a direct relationship between plasma antagonist concentrations and effects on CXCL11-induced CXCR3 internalisation.

In conclusion, this plasma compatible CXCR3 internalisation assay allows analysis of the relationship between PK and PD for NBI-74330 and its metabolites and discriminates between different dosing regimens. This assay may provide a useful biomarker to evaluate the role of CXCR3 in inflammatory disease.

Chien et al., 2005. The AAPS Journal. 7(3): Article 55.