Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol18Issue1abst057P.pdf

Downregulation of the αvβ6 integrin can occur via short engagement with high affinity ligands or long engagement with low affinity ligands

E. Gower, A. Wilkinson, R. J. Slack. Fibrosis DPU, Respiratory TAU, GlaxoSmithKline, Stevenage, United Kingdom.

Introduction: The arginyl-glycinyl-aspartic acid (RGD) integrin alpha-v beta-6 (αvβ6) has been identified as playing a key role in the activation of transforming growth factor-β (TGFβ) that is hypothesised to be pivotal in the development of fibrosis and other diseases [1]. In the process of identifying αvβ6 integrin inhibitors for the treatment of idiopathic pulmonary fibrosis, a novel mechanism of action for sustained TGFβ inhibition was identified.

Methods: In this study αvβ6 small molecule inhibitors were characterised in a range of pre-clinical *in vitro* (radioligand binding [1], flow cytometry [2], functional TGFβ [3] and high content screening assays) systems. All washout experiments were completed following a 1 h inhibitor pre-treatment. All data shown at least four individual experiments with mean ± SD shown. Half-life ($t_{1/2}$) values were generated from global fitting of all available data sets.

Results: GSK3008348 [1] exhibited high αvβ6 affinity (K_i= 0.02±0.01nM) and a slow dissociation profile ($t_{1/2}$= 7h), whereas SC-68448 [2] demonstrated a low affinity (K_i= 2.0±0.7nM) and a fast dissociation profile ($t_{1/2}$ = 0.1h). Normal human bronchial epithelial (NHBE) cells treated with GSK3008348 or SC-68448 resulted in a concentration-dependent decrease in αvβ6 on the plasma membrane due to rapid internalisation. After washout of 1μM SC-68448, the αvβ6 cell surface re-population $t_{1/2}$= <3h, whereas post washout of 0.1μM GSK3008348 the $t_{1/2}$= 11h. Total β6 was reduced in NHBEs following washout of 0.1μM GSK3008348, whereas SC-68448 was only able to reduce total β6 if present continuously. Release of active TGFβ from NHBEs was inhibited by 0.1μM GSK3008348 and 1μM SC-68448. After washout of SC-68448, release of active TGFβ was restored, whereas after washout of GSK3008348 the inhibition of TGFβ was maintained but was reversible in the presence of 10μM chloroquine (lysosomal inhibitor).

Conclusion: In conclusion, these observations combined suggest the αvβ6 integrin can be degraded as a result of high affinity RGD-binding whereby sustained activation of the integrin intracellularly potentially sorts it for lysosomal degradation rather than direct recycling to the cell surface. In addition, αvβ6 can also be downregulated following sustained engagement of RGD-binding sites with low affinity ligands that do not sort the integrin for immediate lysosomal degradation.

References:

1. Hall ER *et al.*, (2016). *Biochem Pharm* **117**:88-96.
2. Slack RJ *et al.*, (2016). *Pharmacology* **97**:114-125.
3. Xu MY *et al.*, (2009) *Am J Pathol* **174**:1264-1279.