Binding kinetics of a novel muscarinic antagonist, [^{3}H]GSK573719: A comparison to [^{3}H]tiotropium.

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GSK573719 is a potent, pan-active muscarinic cholinergic receptor (mAChR) antagonist that demonstrates slow functional reversibility *in vitro* and long duration of action *in vivo* when administered directly to the lungs. GSK573719 may be suitable for use as a once-daily anti-bronchoconstrictive agent for COPD and is being progressed into humans. In this report (funded by GlaxoSmithKline) we describe the pharmacological characterisation of the binding kinetics of [^{3}H]GSK573719 and [^{3}H]tiotropium to the human mAChR subtype M_2 or M_3 stably expressed in chinese hamster ovary (CHO) cells.

Radioligand binding experiments were conducted using either [^{3}H]GSK573719 or [^{3}H]tiotropium exposed to membrane fragments obtained from CHO cells expressing the human recombinant M_2 or M_3 mAChR at 37°C in binding buffer (50mM HEPES, pH 7.4). Non-specific binding was determined by addition of 10µM atropine. Saturation, association, and dissociation binding studies were performed to determine receptor binding kinetics at the M_2 and M_3 mAChR (equilibrium dissociation constant (K_D), total number of receptors (B_{max}), association rate (k_{on}), dissociation rate (k_{off}), and dissociation half-life ($t_{1/2}$)). Dissociation was initiated by a 1:20 dilution in binding buffer (containing 10µM atropine). All data shown are mean ± SEM, n=4.

Radioligand	**M_2 pK_D**	**M_2 B_{max} (pmol/mg)**	**M_3 pK_D**	**M_3 B_{max} (pmol/mg)**
[^{3}H]GSK573719	9.79 ± 0.08	2.53 ± 0.25	10.5 ± 0.01	5.01 ± 0.10
[^{3}H]tiotropium	10.3 ± 0.08	1.98 ± 0.04	10.7 ± 0.07	3.93 ± 0.15

Table 1. The receptor saturation binding parameters for [^{3}H]GSK573719 and [^{3}H]tiotropium at human M_2 and M_3 receptors.

Specific binding data from saturation experiments were fitted to a one affinity site model and this analysis resulted in pK_D and B_{max} values shown in Table 1. [^{3}H]GSK573719 and [^{3}H]tiotropium both exhibited sub-nM affinity for M_2 and M_3 mAChRs. A comparable affinity for the M_3 receptor was exhibited for both these radioligands whilst [^{3}H]GSK573719 had a marginally greater selectivity for the M_3 over the M_2 receptor (~5-fold) compared with [^{3}H]tiotropium (~3-fold). The B_{max} values for [^{3}H]GSK573719 and [^{3}H]tiotropium at both the human M_2 and M_3 mAChR were similar suggesting that both radioligands were labelling the same population of receptors.

Radioligand	**M_2 k_{on} $M^{-1}\cdot min^{-1}$**	**M_2 k_{off} min^{-1}**	**M_2 $t_{1/2}$ min**	**M_3 k_{on} $M^{-1}\cdot min^{-1}$**	**M_3 k_{off} min^{-1}**	**M_3 $t_{1/2}$ min**

[^{3}H]GSK573719	2.22 ± 0.11 x 10^9	0.074 ± 0.004	9.4 ± 0.5	5.67 ± 0.45 x 10^8	0.0089 ± 0.0012	82.2 ± 11.3
[^{3}H]tiotropium	1.26 ± 0.10 x 10^9	0.023 ± 0.008	39.2 ± 9.7	4.09 ± 0.55 x 10^8	0.0026 ± 0.0003	272.8 ± 27.6

Table 2. The receptor binding kinetic parameters for [^{3}H]GSK573719 and [^{3}H]tiotropium at human M_2 and M_3 receptors.

Comparable values of k_{on} were obtained for the two radioligands at each receptor subtype although [^{3}H]GSK573719 and [^{3}H]tiotropium associated more rapidly with the M_2 mAChR than with the M_3 mAChR (Table 2). [^{3}H]GSK573719 and [^{3}H]tiotropium dissociated faster from the M_2 mAChR than the M_3 mAChR, however dissociation of [^{3}H]GSK573719 from the M_2 mAChR was faster (~4-fold) than that of [^{3}H]tiotropium. The $t_{1/2}$ values for dissociation of [^{3}H]tiotropium were much longer at both receptors.

In summary, [^{3}H]GSK573719 exhibited a high affinity for both the M_2 and M_3 mAChR with a marginally greater selectivity for M_3 over the M_2 sub-type when compared with [^{3}H]tiotropium. The slow dissociation kinetics of [^{3}H]GSK573719 at the M_3 mAChR are consistent with a long duration of action.