available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Allosteric effects of antagonists on signalling by the chemokine receptor CCR5

Ben Haworth [a], Hong Lin [a], Mark Fidock [b], Pat Dorr [b], Philip G. Strange [a,*]

[a] School of Pharmacy, University of Reading, Whiteknights, Reading RG6 6AJ, UK
[b] Discovery Biology, Pfizer GRD, Sandwich Laboratories, Kent CT13 9NJ, UK

ARTICLE INFO

Article history:
Received 23 April 2007
Accepted 20 June 2007

Keywords:
Chemokine receptor (CCR5)
Chemokines
[^{35}S]GTPγS binding
Antagonists
HIV
Receptor internalisation

ABSTRACT

Antagonists of the chemokine receptor, CCR5, may provide important new drugs for the treatment of HIV-1. In this study we have examined the mechanism of action of two functional antagonists of the chemokine receptor CCR5 (UK-396,794, UK-438,235) in signalling and internalisation assays using CHO cells expressing CCR5. Both compounds were potent inverse agonists versus agonist-independent [^{35}S]GTPγS binding to membranes of CHO cells expressing CCR5. Both compounds also acted as allosteric inhibitors of CCL5 (RANTES) and CCL8 (MCP-2)-stimulated [^{35}S]GTPγS binding to CHO-CCR5 membranes, reducing the potency and maximal effects of the two chemokines. The data are consistent with effects of the allosteric inhibitors on both the binding and signalling of the chemokines. Both compounds inhibited CCR5 internalisation triggered by chemokines. When CHO-CCR5 cells were treated with either of the two compounds for prolonged periods of time (24 h) an increase (~15%) in cell surface CCR5 was detected.

1. Introduction

The G protein-coupled chemokine receptor, CCR5, was originally characterised as a receptor responding functionally to the CC-chemokines CCL3 (MIP-1α), CCL4 (MIP-1β) and CCL5 (RANTES) [1]. CCR5 was subsequently described as the primary co-receptor for macrophage tropic human immunodeficiency virus type 1 (HIV-1) [2–5].

Chemokine interaction with CCR5 initiates several events. Activated receptor associates with G proteins, leading to activation of signalling processes, e.g. changes in Ca^{2+} followed by receptor phosphorylation by G protein coupled receptor kinases (GRKs) [6]. This results in association of β-arrestin with the receptor [7] and desensitisation via uncoupling of receptor and G protein. A number of CC chemokines (CCL3, CCL4, CCL5, CCL8, CCL13 and CCL7) bind to CCR5 with different affinities and abilities to activate the receptor [8]. These chemokines can be divided in two subgroups based on amino acid sequence identity [9,10]. CCL3, CCL4 and CCL5 form one subgroup and are full agonists, whereas CCL7, CCL8 and CCL13 form a second subgroup, which share ~60% amino acid identity within the group and ~30% identity with CCL3, CCL4 and CCL5. The two groups of chemokine exhibit differential abilities to activate signalling systems. For example, whereas CCL5 and CCL8 can both stimulate [^{35}S]GTPγS binding, CCL5 will cause internalisation of CCR5 but CCL8 is unable to do so [7,11], suggesting that the two groups of chemokines may stabilise different conformations of CCR5.

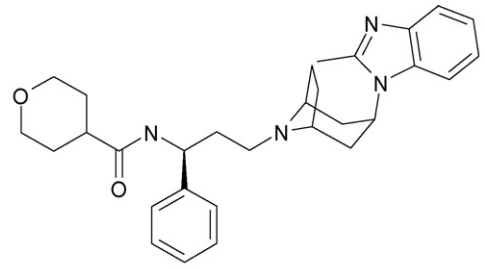
* *Corresponding author*. Tel.: +44 118 378 8015; fax: +44 118 931 0180.
E-mail address: P.G.Strange@rdg.ac.uk (P.G. Strange).

Abbreviations: CHO, cells, Chinese hamster ovary cells; FCS, foetal calf serum; FITC, fluorescein isothiocyanate; GPCR, G protein coupled receptor; GRK, G protein coupled receptor kinase; HIV, human immunodeficiency virus; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PBS, phosphate buffered saline; RANTES, regulated on activation, normal T cell expressed and secreted

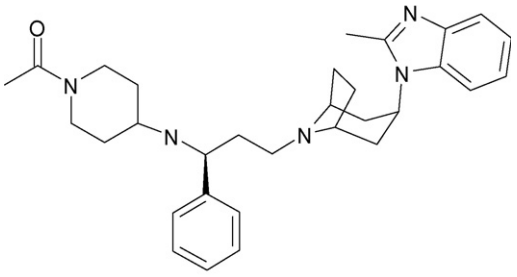
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2007.06.032

The role of CCR5 as a co-receptor for the entry of HIV-1 into cells has prompted the development of small molecule antagonists of CCR5 as a potential therapy for preventing infection by HIV-1 [12]. Several such compounds have been described including 873140 (aplaviroc) [13], UK 427,857 (maraviroc) [14], SCH 351125 (vicriviroc) [15] and TAK 779 [16] and some biochemical studies on these compounds have been reported. The emerging data suggest that the compounds may act as allosteric inhibitors of chemokine binding and function at CCR5 and some differential effects of compounds on the actions of chemokines have been reported. For example, 873140 inhibited CCL3 and CCL5-induced Ca^{2+} responses via CCR5 but did not inhibit CCL5 binding to CCR5 [13]. These small molecule antagonists may also stabilise different conformations of CCR5 accounting for the differential antiviral spectrum of different antagonists [14] and the lack of cross-resistance seen for these different antagonists [17].

In order to probe this further we have analysed the interaction of two small molecule antagonists directed at CCR5 (UK-396,794, UK-438,235, Fig. 1) for their effects on the actions of two chemokines (CCL5 and CCL8) which have previously been shown to interact with CCR5 in a different manner [7,11]. We have examined the interactions between the small molecule drugs and chemokines at CCR5 by determining effects on chemokine-stimulated [^{35}S]GTPγS binding to membranes of CHO cells expressing CCR5 (CHO-CCR5 cells) and by determining effects on chemokine-induced internalisation of CCR5 in CHO-CCR5 cells.



UK-396,794



UK-438,235

Fig. 1 – Structures of UK-396,794 and UK-438,235.

2. Materials and methods

2.1. Cells and materials

CHO cells stably expressing CCR5 (CHO·CCR5) were grown as described previously [7,11]. [^{35}S]GTPγS (~37 TBq mmol^{-1}) was from Amersham Biosciences (Buckinghamshire, UK). CCL5 and CCL8 were purchased from PeproTech (Rocky Hill, NJ). Secondary antibodies were obtained from Sigma (Poole, UK), anti-CCR5 antibodies HEK/1/85a/7a have been described previously [7,11] and all other chemicals were from Sigma (Poole, UK).

2.2. Membrane preparation

Membranes were prepared from confluent cells. Cells were harvested in ice-cold HEPES buffer (20 mM HEPES, 6 mM $MgCl_2$, 1 mM EDTA and 1 mM EGTA, pH 7.4) and homogenized with an ultra-turrax (4× 4 s). The cell debris was collected by centrifugation for 10 min at 1700 × *g*, the resulting supernatant was centrifuged for 1 h at 48,000 × *g* at 4 °C and the pellet resuspended in HEPES buffer, aliquoted and stored at −70 °C. Protein levels were determined using the method of [18] with bovine serum albumin as a standard.

2.3. [^{35}S]GTPγS-binding assays

The [^{35}S]GTPγS-binding assays were carried out essentially as described [11]. Cell membranes (5 μg) were incubated in triplicate in [^{35}S]GTPγS-binding buffer containing 20 mM HEPES, 100 mM NaCl, 10 mM $MgCl_2$ and 0.1% BSA, pH 7.4 and GDP (1 μM for agonist/antagonist assays, 0.1 μM for inverse agonist assays) in a final volume of 180 μl in the absence or presence of a chemokine (at the concentration stated) to measure the basal and stimulated nucleotide exchange, respectively. Antagonist was also present in this incubation where stated. The reaction was initiated by the addition of cell membranes and the tubes were incubated at 30 °C for 30 or 240 min where stated. After this pre-incubation 20 μl of [^{35}S]GTPγS was added to give a final concentration of 100 pM. The assay was incubated for a further 30 min before termination by rapid filtration through GF/C filters with four washes of 1 ml of ice-cold PBS using a Brandel cell harvester (Gaithersburg, MD). The filters were soaked for at least 6 h in 2 ml of Ultima Gold XR scintillation fluid (Perkin Elmer, Beaconsfield, UK) after which bound radioactivity was determined by liquid scintillation counting.

2.4. Internalisation assay and flow cytometry analysis

CHO·CCR5 cells were incubated with serum-free medium for 2 h at 37 °C, harvested with 2 mM EDTA/PBS and then resuspended in medium without serum at 5 × 10^6 cells/ml. Cells were then incubated with CCL5 (50 nM) for different times in the absence or presence of 100 nM antagonist at 37 °C, and washed in ice cold PBS containing 1% FCS and 1% NaN_3 for flow cytometry analysis. Cell surface expressed CCR5 was detected by flow cytometry using anti-CCR5 antibody HEK/1/85a/7a and fluorescein isothiocyanate (FITC) conjugated anti-rat IgG. Cells were incubated for 1 h at room temperature with

HEK/1/85a/7a (saturating amounts of hybridoma supernatant), washed three times with PBS buffer containing 1% FCS and 1% NaN_3 and incubated for 1 h with FITC labelled anti-rat IgG. Samples were quantified on a FACScan and data analysed with CellQuest software version 3.1 (Becton Dickinson, San Jose, CA). For each experiment, for example, when examining the effects of a chemokine on cell surface CCR5, the relative CCR5 surface expression (%) was calculated as 100 × [mean channel of fluorescence (chemokine treated cells) – mean channel of fluorescence (negative control cells)]/[mean channel of fluorescence (cells treated with medium without serum) – mean channel of fluorescence (negative control cells)]. CHO cells not expressing CCR5, as well as irrelevant monoclonal antibodies were used for negative controls with similar results.

In another set of experiments, CHO-CCR5 cells were cultured for 24 h in the absence or presence of antagonist (100 nM) and cell surface CCR5 was determined using flow cytometry as described above.

2.5. *Data analysis*

Data were analysed using GraphPad Prism (GraphPad Software, San Diego, CA). Concentration/response curves for CCL5 and CCL8 in [^{35}S]GTPγS binding assays were fitted well by models assuming a Hill coefficient of 1. The maximal effects of CCL5 or CCL8 in the absence or presence of antagonists were determined from these analyses. The reduction in the maximal response to the chemokines was calculated as [maximal response in the absence of antagonist – maximal response with highest concentration of antagonist]/[maximal response in the absence of antagonist].

Statistical analyses were performed using Student's *t*-test with $P < 0.05$ or using one-way ANOVA with Bonferroni's multiple comparison as a post-test. Data represent the mean ± S.E.M. of at least three independent experiments.

3. Results

3.1. *Effects of UK-396,794 and UK-438,235 on agonist-independent [^{35}S]GTPγS binding*

Fig. 2 shows the effects of UK-396,794 and UK-438,235 on [^{35}S]GTPγS binding to membranes of CHO cells expressing CCR5 (CHO-CCR5 cells). The two compounds were both potent inverse agonists (pIC_{50} ± S.E.M. UK-438,235 8.71 ± 0.05, UK-396,794 8.93 ± 0.09) with UK-396,794 giving a slightly larger inhibition of agonist-independent [^{35}S]GTPγS binding in this preparation (UK-438,235 −27.9 ± 0.6%, UK-396,794 −30.4 ± 1.5%, $P < 0.05$).

3.2. *Effects of UK-396,794 and UK-438,235 on CCL5- and CCL8-stimulated [^{35}S]GTPγS binding*

Next we analysed the effects of different concentrations of the compounds on the stimulation of [^{35}S]GTPγS binding by CCL5 and CCL8. In the first set of experiments, the chemokines and UK-396,794 or UK-438,235 were pre-incubated together with membranes of CHO-CCR5 cells for 30 min before adding [^{35}S]GTPγS to initiate the binding reaction. Fig. 3 shows the effect of UK-438,235 on CCL5 stimulation of [^{35}S]GTPγS binding. Increasing concentrations of UK-438,235 caused a shift in the CCL5 concentration/response curve to lower potency, up to 10-fold. UK-438,235 also reduced basal [^{35}S]GTPγS binding by about 25% at the highest concentration

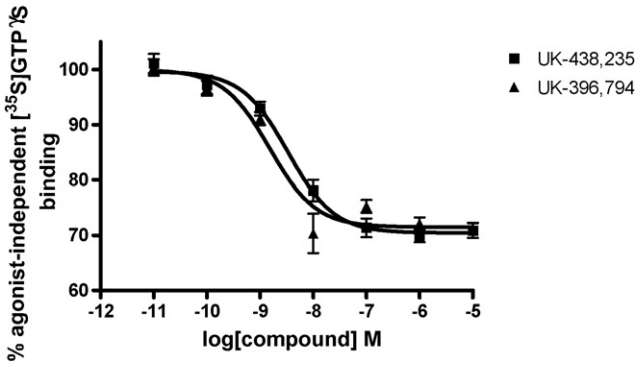


Fig. 2 – Inhibition of agonist-independent [^{35}S]GTPγS binding by UK-438,235 and UK-396,794. Agonist-independent [^{35}S]GTPγS binding to membranes of CHO-CCR5 cells was determined in the presence of increasing concentrations of antagonists as described in Section 2. Data are given as mean ± S.E.M. and are from a representative experiment replicated as in the text.

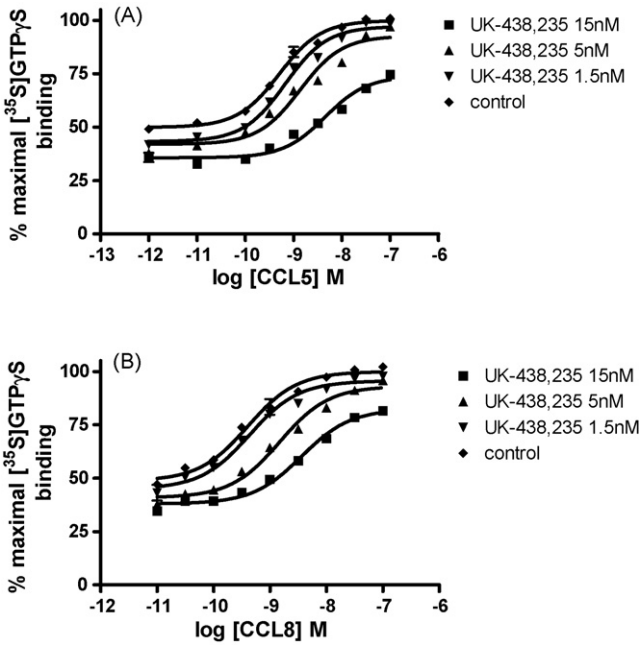


Fig. 3 – Effects of UK-438,235 on chemokine stimulation of [^{35}S]GTPγS binding to membranes of CHO-CCR5 cells. The effects of CCL5 (panel A) and CCL8 (panel B) to stimulate [^{35}S]GTPγS binding were determined in the presence of increasing concentrations of UK-438,235 as described in Section 2. Chemokine and UK-438,235 were pre-incubated with CHO-CCR5 membranes for 30 min. Data are expressed as a percentage of the maximum [^{35}S]GTPγS binding stimulated by CCL5 or CCL8 and are given as mean ± S.E.M. from a representative experiment repeated at least three times with similar results.

Table 1 – Effects of antagonists on chemokine signalling at CCR5 expressed in CHO-CCR5 cell membranes

	Incubation time (min)	CCL5		CCL8	
		Reduction in maximum response (%)	Log dose ratio	Reduction in maximum response (%)	Log dose ratio
UK-438,235	30	14.0 ± 4.3	1.00 ± 0.07	15.1 ± 3.0	0.81 ± 0.07
UK-396,794	30	26.9 ± 0.9	1.27 ± 0.08	23.8 ± 2.4	0.94 ± 0.05
UK-438,235	240	28.8 ± 1.8	0.63 ± 0.02	–	–
UK-396,794	240	44.0 ± 4.1	1.36 ± 0.13	–	–

The effects of CCL5 and CCL8 to stimulate [^{35}S]GTPγS binding were determined in the presence of increasing concentrations of UK-438,235 or UK-396,794 as described in Figs. 3 and 4. Concentration/response curves for the two chemokines were obtained in the presence of different concentrations of either antagonist. The % reduction in maximal chemokine response is given for 15 nM antagonist calculated as described in Section 2. The potency (EC_{50}) for the chemokine was determined and data are given for the log dose ratio for 15 nM antagonist. The log dose ratio is defined as (pEC_{50} (control) − pEC_{50} (with antagonist)). Data are shown where chemokine and antagonist were pre-incubated with CHO-CCR5 membranes for 30 or 240 min. Values are given as mean ± S.E.M. for three or more experiments.

of drug used and there was also a reduction in the maximum effect of CCL5 (~15%), although this was somewhat variable. Similar data are seen with CCL8 and for this chemokine there is a clear reduction in the maximum effect in all experiments at the higher concentrations of UK-438,235. Taken together, these data provide evidence that UK-438,235 is acting in a non-competitive manner to reduce both the potency and efficacy of the chemokines. Effects were similar for both chemokines. A summary of the effects of the antagonists on the potency of chemokines and their maximal responses is given in Table 1.

UK-396,794 behaved in a similar manner versus CCL5 and CCL8 in that it reduced both the potency and maximal effects of the chemokines to stimulate [^{35}S]GTPγS binding (Fig. 4, Table 1). It also reduced the basal [^{35}S]GTPγS binding. There was a tendency for the effect on maximal [^{35}S]GTPγS binding to be greater (~25%) as compared to the effects of UK-438,235. As for UK-438,235 these experiments provide evidence for non-competitive effects between UK-396,794 and CCL5 and CCL8.

We were concerned that equilibrium might not have been reached between the chemokines and the UK compounds. All CCR5 functional antagonists tested to date show prolonged functional occupancy of CCR5 in vitro [17]. More specifically with respect to the compounds used in this study, maraviroc has been reported to dissociate slowly from CCR5 ($t_{1/2}$ = 16 h [19]), highlighting the difficulty in establishing equilibrium conditions. Slow kinetics of reversal of CCR5 blockade have also been reported in [13] for maraviroc and other CCR5 antagonists. Preliminary studies have also shown that UK-396,794 and UK-438,235 dissociate slowly from CCR5 under the

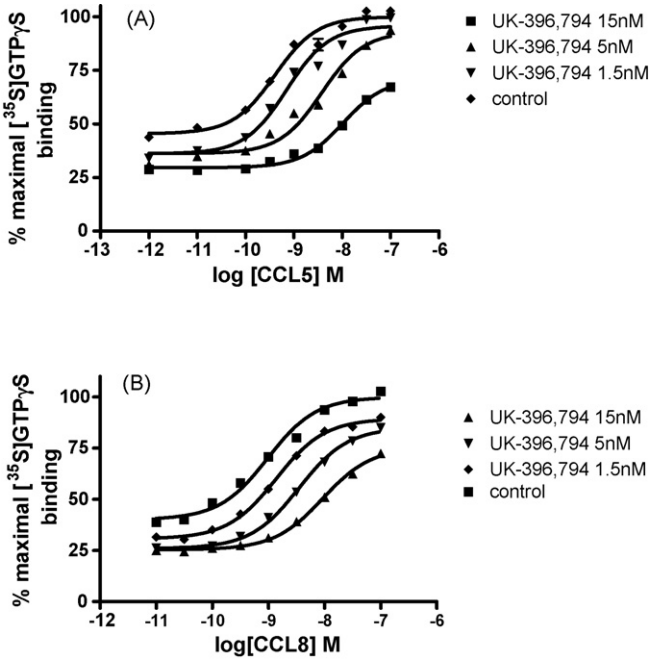


Fig. 4 – Effects of UK-396,794 on chemokine stimulation of [^{35}S]GTPγS binding to membranes of CHO-CCR5 cells. The effects of CCL5 and CCL8 to stimulate [^{35}S]GTPγS binding were determined in the presence of increasing concentrations of UK-396,794 as described in Section 2. Chemokine and UK-396,794 were pre-incubated with CHO-CCR5 membranes for 30 min. Data are expressed as a percentage of the maximum [^{35}S]GTPγS binding stimulated by CCL5 or CCL8 and are given as mean ± S.E.M. from a representative experiment repeated at least three times with similar results.

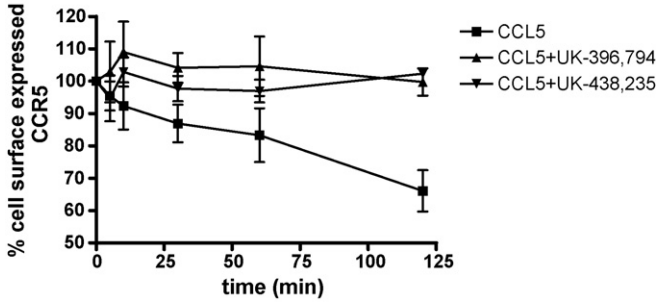


Fig. 5 – Effect of antagonists on CCL5-induced internalisation of CCR5 in CHO-CCR5 cells. Cell surface CCR5 was determined using flow cytometry and an anti-CCR5 antibody as described in Section 2. Cells were treated with 50 nM CCL5 alone and in the presence of 100 nM UK-438,235 or UK-396,794.

same conditions as reported for maraviroc, with $t_{1/2}$ values of 10 and 1 h, respectively (P. Dorr unpublished, see also [17,20]). Further experiments were, therefore, performed with a 240 min pre-incubation time for the chemokine, UK-396,794 or UK-438,235 and receptor before addition of the [^{35}S]GTPγS. For UK-438,235 its effects are very similar to those seen after a 30 min pre-incubation, although the inhibition of maximal [^{35}S]GTPγS binding was clearer (~30%) (Table 1). For UK-396,794, however, effects on the potency of chemokines were similar after the longer incubation but the reductions in the maximal effects of CCL5 to stimulate [^{35}S]GTPγS binding were greater (~44%) (Table 1). Experiments conducted with longer pre-incubation times than 240 min gave similar data (data not shown).

3.3. *Effects of UK-396,794 and UK-438,235 and chemokines on cell surface levels of CCR5 in CHO-CCR5 cells*

CCL5-induced internalisation of CCR5 in CHO-CCR5 cells was assayed using flow cytometry. Whereas treatment of CHO-CCR5 cells with CCL5 (50 nM) lead to about 35% internalisation of CCR5 after 2 h, this effect was blocked completely in the presence of 100 nM of either UK-396,794 or UK-438,235 (Fig. 5). The level of CCR5 in the presence of 100 nM UK-396,794 or UK-438,235 was similar to control levels after 2 h but when cells were treated for 24 h with the compounds there was an elevation of cell surface CCR5 when compared to parallel controls (15.7 ± 3.1% (UK-396,794), 18.7 ± 6.8% (UK-438,235), mean ± S.E.M., six experiments). The effects of both drugs were significant ($P < 0.05$, paired *t*-test)

4. Discussion

In this study we have examined the effects of two small molecule inhibitors of the chemokine receptor CCR5. Both compounds were potent inverse agonists, inhibiting agonist-independent activation of CCR5 by about 30% in this preparation as assessed by [^{35}S]GTPγS binding. This shows that CCR5 is constitutively active in this preparation assessed using this assay system. We examined this apparent constitutive activity further by carrying out chronic treatment of CHO-CCR5 cells with the two inhibitors. A 24 h treatment with either compound lead to an up-regulation of cell surface CCR5 in the cells. There was no up-regulation upon short-term treatment. It has been suggested that such up-regulation is a property inherent to inverse agonists [21] and our data are consistent with this idea. Indeed for other receptors, compounds exhibiting inverse agonism cause up regulation of their cognate receptor [21]. This is likely to be a widespread phenomenon given that inverse agonism has been widely described for compounds previously labelled as antagonists [22]. For other CCR5 antagonists, data have been published showing inverse agonism [23–25], suggesting that this is a general property of such compounds directed at CCR5 which may have implications for their therapeutic use.

Both compounds also inhibited effects of chemokines at CCR5, e.g. chemokine-induced internalisation of CCR5 and chemokine-stimulated [^{35}S]GTPγS binding to membranes of CHO-CCR5 cells. Chemokine (CCL5, CCL8) concentration/response curves for stimulation of [^{35}S]GTPγS binding were obtained in the presence of increasing concentrations of antagonist. Concentration/response curves were progressively shifted to higher concentrations by the antagonists and there was a suppression of the lower and upper asymptotes of the curves. Suppression of the lower asymptote is a reflection of the inverse agonism of the compounds, whereas suppression of the upper asymptote suggests non-competitive effects of the drugs. Indeed the shift in the EC_{50} of the chemokine together with the suppression of the upper asymptote are consistent with non-competitive effects of the drugs with the drug affecting both the maximal functional effect of the chemokine and its binding affinity [26]. When the effects of the antagonists on the two chemokines (CCL5 and CCL8) were compared no differences were seen. Therefore, although these two chemokines have different abilities to influence signalling systems [11] this is not seen in the effects of the antagonists on the responses to these two agonists. In control experiments, high concentrations of antagonist inhibited chemokine-stimulated [^{35}S]GTPγS binding completely and to the same level irrespective of the chemokine concentration (data not shown). This shows that even though the antagonists are acting allosterically there is no ceiling to their effects when acting against a chemokine agonist and complete inhibition of chemokine signalling is achieved at high concentrations of antagonist.

The data obtained in the present study are consistent with non-competitive effects of these antagonists on chemokine

binding and signalling via CCR5 as has been described for other CCR5 antagonists. The suppression of the maximal chemokine effect in [^{35}S]GTPγS binding assays could also be accounted for if the actions of the antagonists were slowly reversible and indeed it has been shown that some of these antagonists do dissociate very slowly from the receptor. This would result in prolonged occupancy of receptors by antagonist during chemokine stimulation. This has been referred to as a hemi-equilibrium and could lead to apparent suppression of the maximal response [27]. In such a situation, however, extended incubation times should lead to a reduction in the effect of the antagonist. When such extended incubation times were tested in the present study, the effects of one of the compounds (UK-396,794) were accentuated. It seems, therefore, that in the present study the suppression of the maximal chemokine effect is not due to slowly reversible antagonism and there may be complex effects of the chemokine on the kinetics of antagonist effects.

We also estimated the dissociation constant for the two antagonists using the Gaddum equation and double reciprocal plots of equi-effective concentrations of agonist in the presence and absence of the antagonist [27]. The use of this approach here is complicated by the suppression of the basal response but estimates were obtained using the effects of 5 nM of each antagonist where suppression of basal is usually incomplete. Based on the data shown in Figs. 3 and 4 the K_B for UK-396,794 and UK-438,235 were ~0.25 and ~0.8 nM, respectively.

The actions of these antagonists may, therefore, be summarised by the model shown in Fig. 6. In this model, chemokine signalling occurs via the agonist/receptor (AR) complex. When the allosteric inhibitor (I) binds, the resultant complex (ARI) is inactive accounting for the progressive reduction in the maximal effects of agonists. The decrease in potency of the agonists results from a combination of effects on signalling and on agonist affinity in the ARI complex.

It is important to understand the implications of the data described in this report. The CCR5 antagonists studied here are in fact inverse agonists that can allosterically inhibit the effects of chemokines acting at CCR5. In acute assays the compounds will inhibit the effects of chemokines at this receptor. In assays based on the chronic treatment of recombinant cells expressing CCR5 with compounds such as UK-396,794 or UK-438,235, the inverse agonist nature of the compounds is usually associated with receptor up-regulation. This is a form of tolerance and, if it occurs in vivo, could lead to CCR5 over-stimulation upon withdrawal of the CCR5 inverse agonist drugs. Increased expression of CCR5 has indeed been reported following vicriviroc treatment in native cell cultures (peripheral blood lymphocytes [28]), albeit to a low extent, and likely to be influenced by compound-mediated reduction in internalisation. Evidence from administration to humans in clinical development programmes for maraviroc has indicated no compound effects on CCR5 expression levels [29] or downstream immune effects associated with enhanced CCR5 signalling following withdrawal of any dosing regime undertaken to date.

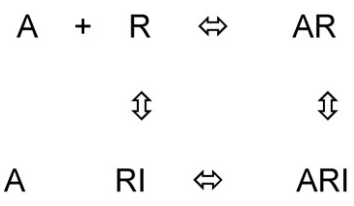


Fig. 6 – Model for actions of CCR5 antagonists. The model shows the agonist (A) and the antagonist (I) interacting with the receptor (R) as described in the text.

Acknowledgement

We thank Pfizer GRD-Sandwich Laboratories for financial support and for supplying reagents.

REFERENCES

[1] Samson M, Labbe O, Mollereau C, Vassart G, Parmentier M. Molecular cloning and functional expression of a new human CC-chemokine receptor gene. Biochemistry 1996;35:3362–7.
[2] Alkhatib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, Murphy PM, et al. CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 1996;272:1955–8.
[3] Choe H, Farzan M, Sun Y, Sullivan N, Rollins B, Ponath PD, et al. The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. Cell 1996;85:1135–48.
[4] Doranz BJ, Rucker J, Yi Y, Smyth RJ, Samson M, Peiper SC, et al. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell 1996;85:1149–58.
[5] Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, Nagashima KA, et al. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. Nature 1996;381:667–73.
[6] Pitcher JA, Freedman NJ, Lefkowitz RJ. G protein-coupled receptor kinases. Annu Rev Biochem 1998;67:653–92.
[7] Mueller A, Kelly E, Strange PG. Pathways for internalization and recycling of the chemokine receptor CCR5. Blood 2002;99:785–91.
[8] Blanpain C, Migeotte I, Lee B, Vakili J, Doranz BJ, Govaerts C, et al. CCR5 binds multiple CC-chemokines: MCP-3 acts as a natural antagonist. Blood 1999;94:1899–905.
[9] Baggiolini M, Dahinden CA. CC chemokines in allergic inflammation. Immunol Today 1994;15:127–33.
[10] Baggiolini M, Dewald B, Moser B. Human chemokines: an update. Annu Rev Immunol 1997;15:675–705.
[11] Mueller A, Mahmoud NG, Goedecke MC, McKeating JA, Strange PG. Pharmacological characterization of the chemokine receptor, CCR5. Br J Pharmacol 2002;135:1033–43.
[12] Fatkenheuer G, Pozniak AL, Johnson MA, Plettenberg A, Staszewski S, Hoepelman AI, et al. Efficacy of short-term monotherapy with maraviroc, a new CCR5 antagonist, in patients infected with HIV-1. Nat Med 2005;11:1170–2.
[13] Watson C, Jenkinson S, Kazmierski W, Kenakin T. The CCR5 receptor-based mechanism of action of 873140, a potent allosteric noncompetitive HIV entry inhibitor. Mol Pharmacol 2005;67:1268–82.

[14] Dorr P, Westby M, Dobbs S, Griffin P, Irvine B, Macartney M, et al. Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. Antimicrob Agents Chemother 2005;49:4721–32.
[15] Palani A, Shapiro S, Josien H, Bara T, Clader JW, Greenlee WJ, et al. Synthesis, SAR, and biological evaluation of oximino–piperidino–piperidine amides. 1. Orally bioavailable CCR5 receptor antagonists with potent anti-HIV activity. J Med Chem 2002;45:3143–60.
[16] Baba M, Nishimura O, Kanzaki N, Okamoto M, Sawada H, Iizawa Y, et al. A small-molecule, nonpeptide CCR5 antagonist with highly potent and selective anti-HIV-1 activity. Proc Natl Acad Sci USA 1999;96:5698–703.
[17] Dorr P, Todd K, Irvine R, Robas N, Thomas A, Fidock M, et al. Site directed mutagenesis studies of CCR5 reveal differences in the interactions between the receptor and various CCR5 antagonists. In: Proceedings of the 45th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy; 2005.
[18] Lowry O, Rosebrough N, Farr A, Randall R. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265–75.
[19] Napier C, Sale H, Mosley M, Rickett G, Dorr P, Mansfield R, et al. Molecular cloning and radioligand binding characterization of the chemokine receptor CCR5 from rhesus macaque and human. Biochem Pharmacol 2005;71:163–72.
[20] Dorr P, Dobbs S, Rickett G, Lewis B, Macartney M, Westby M, et al. In vitro evaluation of prolonged CCR5 occupancy by small molecule antagonists with anti HIV activity. In: Proceedings of the 43rd Annual Interscience Conference on Antimicrobial Agents and Chemotherapy; 2003.
[21] Milligan G, Bond RA. Inverse agonism and the regulation of receptor number. Trends Pharmacol Sci 1997;18:468–74.
[22] Kenakin T. Efficacy as a vector: the relative prevalence and paucity of inverse agonism. Mol Pharmacol 2004;65:2–11.
[23] Dorr P, Macartney M, Rickett G, Smith-Burchnell C, Dobbs S, Mori J, et al. UK-427,857, a novel small molecule HIV entry inhibitor is a specific antagonist of the chemokine receptor CCR5. In: Proceedings of the 10th Conference on Retroviruses and Opportunistic Infections; 2003.
[24] Labrecque J, Anastassov V, Lau G, Darkes M, Mosi R, Fricker SP. The development of an europium-GTP assay to quantitate chemokine antagonist interactions for CXCR4 and CCR5. Assay Drug Dev Technol 2005;3:637–48.
[25] Strizki JM, Xu S, Wagner NE, Wojcik L, Liu J, Hou Y, et al. SCH-C (SCH 351125), an orally bioavailable, small molecule antagonist of the chemokine receptor CCR5, is a potent inhibitor of HIV-1 infection in vitro and in vivo. Proc Natl Acad Sci USA 2001;98:12718–23.
[26] Ehlert FJ. Analysis of allosterism in functional assays. J Pharmacol Exp Ther 2005;315:740–54.
[27] Kenakin T, Jenkinson S, Watson C. Determining the potency and molecular mechanism of action of insurmountable antagonists. J Pharmacol Exp Ther 2006;319:710–23.
[28] Xu L, Wojcik N, Strizki JM. Antagonism of the CCR5 receptor by SCH-C leads to elevated beta-chemokine levels and receptor expression in chronically treated PBMC cultures. In: Proceedings of the 9th Conference on Retroviruses and Opportunistic Infections; 2002.
[29] Penny M, Myrand S, Lin C, Boucher M, Man M, James I. Pharmacogenetic analysis of polymorphisms in the chemokine receptors CCR5 and CCR2 in the clinical development of a CCR5 antagonist (UK-427,857) for the treatment of HIV/AIDS. In: FDA Science Symposium; 2004.