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COMMENTARY

Internalization: what does it tell us about pharmacokinetic and pharmacodynamic properties of an antagonist?

A Mueller

School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich, UK

Chemokine receptors play an important role in trafficking leukocytes within the body, a process that depends on expression of the receptors on the cell surface. Expression levels are regulated by the rate of internalizing receptor compared to the rate of recycling/recovering receptor. Internalization is commonly induced by binding of agonists to their receptors that in turn use clathrin-coated pits or caveolae to internalize. Joplin and colleagues describe a novel usage of internalization assays to determine pharmacokinetic/pharmacodynamic relationships of an antagonist on CXCR3 in a murine system. Intriguingly their results show that internalization assays give robust data about the pharmacokinetics/pharmacodynamics of different agonists and antagonists in an *in vivo* model. This kind of assay will allow investigations of the pharmacological properties of agonists and antagonists in a completely different setting and also give new insight into the regulation of cell surface expression of chemokine receptors and other G protein-coupled receptors, which can lead to potential novel therapeutic targets.
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Abbreviations: GPCRs, G protein-coupled receptors; PTX, pertussis toxin

Chemokines or chemotactic cytokines are small, basic proteins that bind to chemokine receptors and induce a variety of different cellular responses. Chemokine receptors are G protein-coupled receptors and are best known for their involvement in providing leukocyte trafficking in response to chemokine gradients (Thelen, 2001). Interest in the regulation of chemokine receptor expression on the cell surface has increased since the discovery in the 1990s that HIV-1 uses the chemokine receptors CCR5 and CXCR4 as coreceptors to gain entry into cells. Subsequently, investigations have been carried out into natural occurring CCR5 mutations (CCR5Δ32) that prevent CCR5 expression on the cell surface and provide a certain degree of immunity against HIV-1 infection. We and others have shown that chemokine receptors can be rapidly internalized after activation by agonists (Mueller *et al*., 2002; Mariani *et al*., 2004; Fox *et al*., 2006) and recently several antagonists have been described, which efficiently internalize receptors (Feng *et al*., 2006). In the case of CXCR4 and CCR5 those antagonists might therefore provide novel tools to prevent HIV-1 infections, since downregulation of the receptor expression on the cell surface will render those cells immune against HIV-1 infection. It is now known that different receptors exhibit distinct mechanisms of internalization, for example CXCR3 and CCR4 internalization is independent of pertussis toxin treatment, whereas CCR5 internalization depends on Gαi activation (Sauty *et al*., 2001; Mueller *et al*., 2002). Generally it is agreed that chemokine receptors use the clathrin-coated pit pathway as a default mechanism for internalization. In addition, there is strong evidence that lipid rafts or at least cholesterol in the cell membrane affect internalization of several chemokine receptors (Mueller *et al*., 2002; Mariani *et al*., 2004). However, we still do not know a great deal about receptor expression *in vivo* and what part pharmacokinetic or pharmacodynamic properties of an agonist or antagonist play in an *in vivo* setting.

In this issue of the *British Journal of Pharmacology*, Joplin *et al*. (2007) used internalization assays to analyse the pharmacokinetic/pharmacodynamic relationship of a CXCR3 antagonist in a murine system. In their study, the internalization assays delivered a robust set of data that allowed them to determine the rank order of potency and efficacy of various CXCR3 ligands. Interestingly, they showed that a specific CXCR3 antagonist, NBI-74330, blocks internalization of CXCR3 induced by CXCL11 in a dose-dependent manner with similar affinities to those observed with [^{35}S]-GTPγS-binding assays. Furthermore, plasma samples from mice dosed with the antagonist NBI-74330 showed a time-dependent ability to internalize CXCR3 efficiently from the cell surface in an *ex vivo* setting. This kind of assay will not only provide valuable information for determining the

Correspondence: Dr A Mueller, School of Chemical Sciences and Pharmacy, University of East Anglia, University Plain, Norwich NR4 7TJ, UK.
E-mail: anja.mueller@uea.ac.uk
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optimum dosing-regimes when using an antagonist in clinical situations, but will also impart crucial pharmacological information about how many of the receptors on the cell surface will be blocked and how many can be internalized after employment of this antagonist. Consequently, the conclusion of the paper that this assay distinguishes between dosing regimes for the CXCR3 antagonist leaves us with an exciting new tool to help us understand what effect different antagonists have on receptor expression in an *in vivo* context. As CXCR3 is implicated in rheumatoid arthritis, multiple sclerosis and transplant rejection, the *in vivo* studies described by Joplin *et al*. (2007) are needed to validate potential therapeutic approaches for these complex models of inflammation. In addition to these, there are further applications for these assays. As mentioned above, a small molecule antagonist may well be the drug of choice for preventing HIV-1 infection. Therefore, pharmacokinetic/pharmacodynamic data detailing the kind of effect these antagonists have on CCR5 or CXCR4 expression in an *in vivo* setting will provide vital information for their therapeutic use.

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