Recent advances in secondary pharmacology provide a boost for reducing adverse drug reactions by early in vitro off-target mitigation guided by reverse translation from clinical experience and help to reduce the volume of animal experiments.

Secondary pharmacology: screening and interpretation of off-target activities – focus on translation

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Secondary pharmacology is an essential component of drug discovery and is used extensively in the pharmaceutical industry for achieving optimal specificity of new drugs via early hazard identification and off-target mitigation. The importance of this discipline has been achieved by increasing its translational value, based on the recognition of biological target–drug molecule–adverse drug reaction (ADR) associations and integration of secondary pharmacology data with pharmacokinetic parameters. Information obtained from clinical ADRs, from recognition of specific phenotypes of animal models and from hereditary diseases provides increasing regulatory confidence in the target-based approach to ADR prediction and mitigation. Here, we review the progress of secondary pharmacology during the past decade and highlight and demonstrate its applications and impact in drug discovery.

Introduction

Since our previous review on *in vitro* safety (secondary) pharmacology [1], the pharmaceutical industry has introduced standard *in vitro* pharmacological profiling approaches to investigate the mode of action and/or effects of molecules not related to their anticipated therapeutic target. As described by the International Conference on Harmonization (ICH) in their Guidance for Industry: S7A Safety Pharmacology Studies for Human Pharmaceuticals, 'ligand binding or enzyme assay data suggesting a potential for adverse effects should be considered in the selection and design of safety pharmacology studies' (ICH S7A: II.B. 3) [2]. Regulatory authorities expect that diligence has been employed to determine specific off-target effects when adverse drug reactions (ADRs) emerge in preclinical safety evaluations, during clinical studies or in the post-marketing phase [3]. This allows the sponsor and health authorities to have a more informed view of the composite mechanism of action of a drug candidate, and a comparative understanding of different drugs within a class.

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We feel that 10 years after our original review of the subject in *Drug Discovery Today*, it is time to follow up on the progress and discuss the strengths and weaknesses of the secondary pharmacology approach. In this review, we highlight the introduction of translational aspects of off-target phenotypes which has put secondary pharmacology in the mainstream of early safety evaluation of drug candidates and had a clear impact on clinical adverse event predictions.

Secondary pharmacology profiling panels have been further refined because several targets and pathways are now well established as contributors to clinical ADRs [1,4,5], and mitigation strategies are introduced in early drug development by testing affinities of compounds at these targets. As discussed in recent publications, the secondary pharmacology approach is amenable for off-target-related risk assessment and mitigation during lead optimization [1,3,5–7] and general criteria have been discussed on the minimum list of those targets that would qualify for the purpose [5]. However, a reasonable industry-regulatory consensus has not been reached yet. The four main criteria used for the assembly of the off-target panels are: (i) established target–ADR association, preferably with clinical proof; (ii) inclusion of targets with high-impact ADRs; (iii) high hit rate targets; and (iv) assay format. Here, we focus on the concept outlined in the ICH S7A: II.B.3 guideline, which covers target-based assays and does not cover the more complex phenotypic approaches.

Guidance for selection of targets

There are several options for determining which targets should be considered in a secondary pharmacology profiling panel. Whereas any target could qualify for off-target selection, it is important that *in vitro* pharmacological effects are translatable to ADRs. The most reassuring way to find out about this correlation is to profile drugs and reference compounds that have well-established clinical safety information from clinical trials and/or post-marketing pharmacovigilance (e.g., FDA Adverse Event Reporting System; FAERS). Targets with acknowledged therapeutic effects (well-evidenced primary target knowledge) are included in secondary pharmacology panels in case they are encountered accidentally and affect pharmacological homeostasis. The association of small molecule drugs with target–ADR pairs must be interpreted carefully because these drugs can interact with several or many undetected off-targets. However, examination of pharmacovigilance data and broad spectrum *in vitro* secondary pharmacology profiling can identify strong associations, in particular when several drugs show the same ADR profile and express similar *in vitro* target characteristics. The best-known examples are hERG inhibition associated with ventricular arrhythmia [8] and serotonin receptor 2B (5-HT_{2B}) agonism associated with cardiac valvular disease (CVD) [9]. However, it has to be noted that the recognition of the risk associated with the above-mentioned targets required several years of investigations. Increasing use of pharmacovigilance and analysis of human genetic disorders help to identify novel target–ADR associations as medicines with new therapeutic targets enter the clinic, for example in the case of protein kinase inhibitors (see below).

Once target–ADR associations had been established, panels for secondary pharmacology profiling were assembled and large-scale efforts generated marketed drug databases with a wealth of information on pharmacological characteristics. These include BioPrint [10], a rapid system for profiling cellular activities [11] and ToxCast [12], databases that greatly supported reverse translation. However, this is not an easy task to achieve; many drugs affect several targets with various potencies which could be associated with the same or similar side effects [13]. This is most evident for two classes of drugs: (i) the first generation of protein kinase inhibitors [14], which bind to the high-homology ATP-binding site; and (ii) many psychiatric drugs with broad G-protein-coupled receptor (GPCR) activity [15], resulting in pharmacological promiscuity and associated complex ADR profiles.

The introduction of monoclonal antibodies (mAbs) as therapeutic treatments did open up the possibility of refining ADR–target associations. Because they are highly specific for their selected targets a one-to-one correlation can be established with their 'treatment phenotype'. One of the best examples is the use of mAbs for protein kinase inhibition, where the majority of small molecular inhibitors tend to be promiscuous. For example treatment with bevacizumab, a recombinant humanized mAb to vascular endothelial growth factor (VEGF), is associated with hypertension [16] and this class effect is observed with other small molecule drugs that, among several other kinase targets, hit the VEGF receptor [17]. However, a recent case highlights that differentiation might be important between mAbs that affect ligands or respective receptors, such as brodalumab [anti-interleukin (IL)17RA] and secukinumab (anti-IL17) which recognize the receptor and the ligand, respectively [18]. In this particular case, increased incidence of suicidal intent was associated only with brodalumab, the anti-IL17RA mAb, and not with secukinumab, the ligand-specific mAb. To date, there is no clear explanation for this difference; possibilities include involvement of nonspecific IL17 receptor activation in central nervous system (CNS) functions, or as a simple alternative the cases seen during the clinical trials were coincidental [18]. In this case, the absence of a clear mechanistic understanding makes the association inconclusive.

Phenotypes of hereditary diseases also provide important sources of information for target–ADR associations. Two well-known hereditary channelopathies helped to identify the link between ion channels (e.g., hERG) and drug-related cardiac side effects [long QT (LQT)2 syndrome] [19]. However, a strong contribution of the target to developmental pathways might distinguish between the full-blown phenotype of a hereditary disease and a drug-related, acquired effect. As an example, gain-of-function mutations in CACNA1C, the gene encoding the calcium channel $Ca_v1.2$ α subunit, can cause Timothy syndrome which is characterized by increased cellular excitability. The syndrome includes autism, autism spectrum disorder, poor dental enamel coating and cardiac dysfunctions, including LQT8 syndrome, cardiac arrhythmia and heart malformations [20]. However, the effects of pharmacological $Ca_v1.2$ channel blockade are largely restricted to acute cardiovascular effects and other symptoms of Timothy syndrome are associated with the chronic contribution of the channel to developmental pathways.

Occasionally, the unexpected appearance of a highly specific ADR that resembles a syndrome leads to the identification of the off-target. This is the case of the rare incidence of Wernicke encephalopathy in conjunction with the Janus kinase (JAK2) inhibitor fedratinib. The cause of Wernicke encephalopathy is vitamin B1 deficiency as a result of lack of absorption or transport