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Potential functional and pathological side effects related to off-target pharmacological activity

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ABSTRACT

Most pharmaceutical companies test their discovery-stage proprietary molecules in a battery of in vitro pharmacology assays to try to determine off-target interactions. During all phases of drug discovery and development, various questions arise regarding potential side effects associated with such off-target pharmacological activity. Here we present a scientific literature curation effort undertaken to determine and summarize the most likely functional and pathological outcomes associated with interactions at 70 receptors, enzymes, ion channels and transporters with established links to adverse effects. To that end, the scientific literature was reviewed using an on-line database, and the most commonly reported effects were summarized in tabular format. The resultant table should serve as a practical guide for research scientists and clinical investigators for the prediction and interpretation of adverse side effects associated with molecules interacting with components of this screening battery.

1. Introduction

Most pharmaceutical companies test their discovery-stage, proprietary, small molecules in a battery of in vitro molecular pharmacology assays (often in the form of ligand binding) to identify potential off-target interactions. Positive findings in these assays are often followed up by testing for functional activity and to generate more precise data, such as an IC_{50} value (Blomme & Will, 2016). During all phases of drug discovery and development, various questions arise regarding potential side effects associated with off-target pharmacological activity. When such questions need to be addressed, investigators typically quickly review the scientific literature to determine what the most likely side effects are to occur due to association with these off-target interactions. However, the literature is filled with reports of major, minor and seemingly-conflicting effects, and scientists have different degrees of expertise in sifting through publications to decide what is most relevant, especially when time is of essence.

Over the last several years, a handful of excellent review articles have been published summarizing potential adverse effects at various pharmacological targets (e.g., Bowes et al., 2012; Hamon et al., 2009; Whitebread, Hamon, Bojanic, & Urban, 2005). However, the number of targets evaluated has been limited and the effects described have mainly been purely pharmacological, as opposed to also pathological, in nature. Additionally, such reviews generally contain only a single reference for each target, and that reference is often to another review article as opposed to the original paper containing the data cited.

The present literature curation effort was undertaken to produce a table summarizing the most likely activity outcomes at 70 receptors, enzymes, ion channels and transporters that are commonly included in AbbVie's current molecular pharmacology screening battery. The targets contained within this battery were carefully selected, over a period of the last two decades, due to their established links with adverse side effects. The references included for these effects were most often the direct source of that information, although in a few cases review articles were referenced when that was the best source for the data. Our intention was to produce a practical, initial resource for research scientists and clinical investigators for the prediction and interpretation of functional and pathological side effects due to activity of their molecules-of-interest at any of the pharmacological sites within this screening battery.

2. Methods

The electronic database PubMed.gov (U.S. National Library of Medicine, National Institutes of Health; http://www.ncbi.nlm.nih.gov/pubmed), which contains records mainly beginning from the year

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1966, was queried regarding functional and pathological side effects. Our literature searches were conducted over a period of several months, ending in June 2016. Search terms included the following key words or phrases: activation, activator, adverse, agonism, agonist, antagonism, antagonist, function, inhibition, inhibitor, knockout, null, review, safety pharmacology, side effect, and/or toxicology. Additional publications were identified from the reference sections of the retrieved papers. Only English language publications were reviewed.

For each of the pharmacological targets that were researched, the most commonly reported findings were listed, generally in order from most to less common but while also attempting to keep the findings for a particular organ or physiological system grouped together. When summarizing the main organ/systems that were affected, a similar ranking of most to less common was used. Whenever conflicting data were found, a result was reported only when a large majority of the publications described such an effect. For example, for publications regarding convulsant-related effects with the adrenergic α_{2A} receptor, the authors found that the large majority of articles concerning antagonism/inhibition reported a proconvulsant effect (thus, "↑ convulsions" was added to that column of the summary table), while anticonvulsant effects for agonism/activation were reported in only a small minority of the publications (thus, nothing regarding convulsions was added to that column of the summary table). When the direction of an effect was fairly evenly divided between publications, a multi-directional symbol (e.g., "↑/↓") was included. Effects that appeared to be specific to only one species were not listed. In general, data from larger animal species (e.g., humans, non-human primates, and dogs) were more heavily weighted than those from smaller animal species (e.g., rabbits, rats, and mice). However, data from human studies with less selective drugs were less heavily weighted than data from non-human animal studies using more selective molecules. Furthermore, negative side effects were emphasized over positive side effects, and the listings presented mainly pertained to systemic (rather than local) exposures.

Most of the effects that were listed in the summary table can occur after acute (e.g., single) dosing. However, when effects were observed mainly after chronic dosing, those effects were differentiated within the table under the subheading of "Chronic dosing". When sufficient developmental toxicity data was available for a target, those effects were added to the table under the subheading of "Developmental toxicity". Because effects can occur over a range of doses, from pharmacological through toxicological amounts and from single through multiple dosings, it is problematic to concisely delineate the range over which an effect begins and ends, so no such distinction was made within the table (except as mentioned above regarding the "Chronic dosing" subheading).

3. Results

Table 1 lists, in alphabetical order, 70 pharmacological targets with established links to adverse side effects. In the column entitled "Main organs & systems affected", those that were most to less commonly reported in the scientific literature as being affected by selective ligands are recorded from left to right, respectively. The most frequently reported effects are listed under their respective columns for activators (i.e., "Agonism/activation effects") and inhibitors ("Antagonism/inhibition effects") of these targets. It can be assumed that full and partial agonists (just like full and partial antagonists) will have similar effects, but with some differences in the relative degree of the effects.

4. Discussion

As new drugs are discovered, tested pre-clinically and clinically, and then become available as marketed products, there are multiple occasions during this lengthy process when questions may arise regarding side effects. The earliest occurrence is commonly during the exploratory phase of drug discovery when it is decided whether it is worthwhile to investigate a particular pharmacological target or a specific chemical series. At that time, the main question is whether the potential benefits from that particular chemical class will outweigh the potential on-target side effects. If the process moves forward, a number of proprietary molecules are typically then synthesized, tested in a battery of assays for off-target activity, and ranked for further testing. The questions at that stage most often relate to what effects can occur due to any off-target activity that was identified, which of the off-target sites may be better or worse to "hit", and whether an adverse effect observed for a given molecule is likely due to its activity at one of these off-target sites. Consideration of predicted clinical exposure is essential during that stage. If a molecule reaches the stage in which an Investigational New Drug (IND) application (or non-U.S. equivalent) is filed, there are often questions by the regulatory authorities regarding the potential implications of off-target interactions by the compound. Surprisingly, we frequently receive similar questions from regulatory agencies at the New Drug Application (NDA) stage, despite the abundance of human clinical safety data presented. Once a drug has been approved and on the market, if an unexpected adverse event occurs, this can result in a frantic review of the scientific literature to attempt to understand why this effect may have occurred and if it can be associated with the drug's known pharmacological profile. Therefore, from the earliest stages of drug discovery through the medication of large patient populations, it is valuable to have reference sources readily available for investigations into side effects.

Table 1 summarized the most commonly reported changes likely to occur due to activity at 70 pharmacological targets that are generally part of AbbVie's current screening battery employed for high-priority, discovery-stage molecules. At that stage, our goal is to test only those targets with good predictive value for adverse effects rather than attempting to completely determine selectivity, since the latter strategy is impractical as well as essentially impossible even with very large screening panels (Blomme & Will, 2016). For instance, although the dopamine D_5 receptor has been associated with effects such as changes in blood pressure and immunity within some controlled laboratory settings, to the best of our knowledge off-target binding affinity at this particular receptor has never stopped a drug from advancing during clinical trials (Prado, Bernales, & Pacheco, 2013; Zeng, Yang, Asico, & Jose, 2007). Therefore, the dopamine D_5 receptor is not included in our current, general, in vitro screening panel. Nevertheless, during earlier stages of drug discovery, it is common to screen molecules for selectivity in assays specifically chosen based upon the therapeutic target as well as known activity by other molecules of the same pharmacological class or chemical series. For example, if a series of molecules is targeted for a specific dopamine receptor subtype but frequently also binds to some subtypes of phosphodiesterase, a useful strategy is to screen against all known dopaminergic receptor subtypes (including D_5) as well as the phosphodiesterase subtypes that had been commonly affected. This earlier, more tailored screening strategy is an essential step for producing molecules with potent binding affinity for the desired pharmacological target while also funneling out the less potent and less selective compounds before they proceed further, including to a more general in vitro screening battery.

The battery of assays that we are currently using has changed substantially from the one that we began employing, over two decades ago, as a general screen for off-target activity. Since that time, we have periodically adjusted which pharmacological targets are included based upon our experiences, but also as our therapeutic areas and chemical spaces have changed. In addition, we review regulatory agency decisions and clinical trial news to ascertain whether there are important safety issues with any targets that we may have been overlooking; websites such as http://www.fda.gov, http://www.ema.europa.eu, and https://www.clinicaltrials.gov are good sources for such information. Furthermore, as human versions of these targets became available, our battery has switched from testing mostly targets derived from rodent tissue to mostly those from human tissue, which