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# High-throughput identification of off-targets for the mechanistic study of severe adverse drug reactions induced by analgesics☆

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ABSTRACT

Drugs may induce adverse drug reactions (ADRs) when they unexpectedly bind to proteins other than their therapeutic targets. Identification of these undesired protein binding partners, called off-targets, can facilitate toxicity assessment in the early stages of drug development. In this study, a computational framework was introduced for the exploration of idiosyncratic mechanisms underlying analgesic-induced severe adverse drug reactions (SADRs). The putative analgesic-target interactions were predicted by performing reverse docking of analgesics or their active metabolites against human/mammal protein structures in a high-throughput manner. Subsequently, bioinformatics analyses were undertaken to identify ADR-associated proteins (ADRAPs) and pathways. Using the pathways and ADRAPs that this analysis identified, the mechanisms of SADRs such as cardiac disorders were explored. For instance, 53 putative ADRAPs and 24 pathways were linked with cardiac disorders, of which 10 ADRAPs were confirmed by previous experiments. Moreover, it was inferred that pathways such as base excision repair, glycolysis/glyconeogenesis, ErbB signaling, calcium signaling, and phosphatidyl inositol signaling likely play pivotal roles in drug-induced cardiac disorders. In conclusion, our framework offers an opportunity to globally understand SADRs at the molecular level, which has been difficult to realize through experiments. It also provides some valuable clues for drug repurposing.



## Introduction

Adverse drug reactions (ADRs) are unexpected effects that occur during normal chemotherapy. Severe ADRs (SADRs) are normally characterized as requiring hospitalization, prolonging hospitalization, being permanently disabling or fatal (Wilke et al., 2007). According to recent statistics in the "Reports Received and Reports Entered into FAERS by Year" from the Food and Drug Administration (FDA) of USA, the number of reported ADRs increased more than 10% every year from 2005 to 2011 (US FDA, 2011). Another report indicated that serious adverse drug events increased 2.6-fold from 1998 through 2005 and that fetal adverse drug events increased 2.7-fold (Moore et al., 2007). It was estimated that SADRs may cost nearly as much as the drug treatment itself (Ingelman-Sundberg, 2008). SADRs are also one of the major factors that lead to drug development failure. To maximally avoid undesired SADRs, a number of experimental assays and computational toxicology tools have been adopted in early drug discovery stages to filter out candidates with the high potential for ADRs from drug candidate pools (Bender et al., 2007; Kwan et al., 2006; Muster et al., 2008). Unfortunately, many of these toxicity analyses did not result in rules or empirical knowledge that can be reused for further drug safety evaluation. This weakness was caused by poor understanding of the mechanisms underlying SADRs.

The causes of ADRs are complex and vary by case. To summarize, drugs may induce adverse reactions through any combination of the following four broad areas: change of micro-environment (Muster et al., 2008; Valerio, 2009), ligand-receptor interaction (on-target or off-target) (Bender et al., 2007; Liebler and Guengerich, 2005), gene regulation and immune mediation (Ji et al., 2003). Although the general mechanisms by which a drug induces ADRs have been summarized, exact knowledge of how a particular ADR is induced remains unclear.

Investigation of a drug-target profile is an efficient approach to solving various problems in etiology and pharmacology (Lindsay, 2003). For instance, the therapeutic effects of a drug generally result from the interaction of the drug with one or more proteins or nucleic acids (so-called therapeutic targets) that are critical in disease processes. Likewise, adverse reactions to a drug are often induced by undesired interactions of the drug with crucial proteins (off-targets) within physiological pathways other than its therapeutic target(s). Hence, the acquisition of a complete drug-off-target interaction profile can potentially facilitate better understanding of molecular mechanisms underlying ADRs. However,

*Abbreviations:* ADRAPs, ADR-associated proteins; SADRs, severe adverse drug reactions; CDs, cardiac disorders; CAs, cardiac arrhythmias; LDs, lung disorders.



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without prior knowledge, it is difficult for conventional molecular technologies to determine what proteins are involved and, furthermore, how the SADRs are triggered and boosted via protein interaction networks. The recent development of toxicogenomics, adopting high throughput technologies such as gene microarrays, enables researchers to monitor the expression of thousands of genes and proteins simultaneously to detect ADR-associated genes or proteins. Even so, it is still difficult to address these questions because of the difficulty of obtaining enough experimental samples, the high cost and the difficulty of data analysis. Therefore, in this study, a computational framework was introduced to rapidly identify putative off-targets of drugs in a high-throughput manner. Upon these off-targets, the idiosyncratic mechanisms underlying SADRs were investigated in a way of molecular network.

## Methods

*The computational framework*. The framework is composed of four sequential analyses. First, the putative protein targets of analgesics were predicted by simulation of drug–target interactions in a large scale using docking software. This step generated the binding target profiles for analgesics. Second, the common off-targets were determined for the selected SADRs by overlapping the target profiles of analgesics that were reported to induce the SADRs. It was assumed that the common off-targets of selected analgesics may partially answer for their common idiosyncratic SADRs. Third, ADR-pathway associations were built by integrating literature-reported drug-ADR, protein–ADR, and protein–pathway relations. The ADR–pathway association networks were also constructed. Fourth, the putative SADR-associated proteins were identified for the selected SADRs by mapping the common off-targets against the corresponding SADR–pathway association sub-networks. Upon the putative SADR-associated proteins and pathways, diagrams were drawn for better illustration of SADR mechanisms.

*Analgesic drugs and their active metabolites*. In this study, six commonly marketed analgesic drugs, which were most frequently reported in fatal and nonfatal serious events (Moore et al., 2007), were chosen for a mechanistic study. They are oxycodone, fentanyl, morphine, acetaminophen, liquicet (acetaminophen–hydrocodone), and rofecoxib (withdrawn from the market). Their pharmacological properties and molecular structures were derived from the DrugBank database (http://www.drugbank.ca) (Knox et al., 2011). These characteristics were briefly summarized in Table 1.

The pharmacokinetic processes (or ADME) have a direct influence on drug efficacy and toxicity. For example, oxycodone can be extensively metabolized to noroxycodone, oxymorphone, and their glucuronides. Both noroxycodone and oxymorphone were reported to exhibit analgesic activity (Knox et al., 2011). Therefore, not only the drug itself but also its major active metabolites were considered in the ADR mechanism study. The active metabolites of these six analgesics was collected from literatures and listed in Table 1. As the combinatorial analgesic liquicet is metabolized separately in the human body, its toxicity was evaluated by combining independent analyses of acetaminophen and hydrocodone.

*Selection of severe adverse drug reactions*. The ADR information for the analgesic drugs was mainly derived from the DailyMed database (http://dailymed.nlm.nih.gov/dailymed/) as well as the literature. DailyMed is a public database that provides standard, comprehensive and up-to-date FDA-labeled drug information, including adverse reactions and pharmacokinetics. By August 2013, it contains more than 50,000 drugs. The ADR terms of analgesics were standardized before later analyses. In total, 65 distinct ADRs were reported for these six analgesics. Some of them are common to analgesics and potentially fatal, e.g., cardiac disorders. Some are drug-specific, e.g., oxycodone-induced lung disorders. Partial list of the SADRs was given in Supplementary Table 1S.

In this work, only three severe ADRs, cardiac disorders (CDs), cardiac arrhythmias (CAs) and lung disorders (LDs) were selected as representatives for mechanism study under the considerations of: These three ADRs are of general interests, however, severe and sometimes fatal. Of the three SADRs, CDs were reported in all six analgesics treatment; to the contrast, LDs were oxycodone-specific. CAs were included as a subset of CDs and two analgesics (oxycodone and fentanyl) were involved. The selection of these three SADRs represents different situations of drug–SADR relations, which will help to evaluate the performance of the computational framework.

*Identification of putative analgesic–target interactions*. The putative protein targets of a drug (and its metabolites) were identified by simulation of ligand–receptor interaction using the reverse docking software INVDOCK (Chen and Ung, 2002). INVDOCK is a ligand–protein inverse docking algorithm, which conducts a computer-automated search of potential protein targets of a small molecule by attempting to dock it to a cavity of each of these proteins. The target search was carried out in the pool of human and mammal protein structures available in the Protein Structure Bank (PDB) (http://www.rcsb.org) by September 2012 (Rose et al., 2013). The putative protein targets were determined in basis of a scoring scheme which performed competitive binding analysis in addition to the evaluation of molecular mechanics ligand–protein interaction energy (Chen and Zhi, 2001). An empirical threshold score of smaller than −20 was adopted for positive assignment of drug–target interactions. To deal with the redundancy of PDB structures, all docked proteins were mapped to their human homologs, and only one representative remained. The putative drug–target interactions were further validated by seeking experimental evidences in public databases like BindingDB and ChEMBL. The BindingDB (http://www.bindingdb.org/bind/index.jsp) is a free database of measured bindingn affinities of ligand–protein interactions (Liu et al., 2007). Currently, BindingDB contains more than 1 million binding data for 6910 protein targets and 421,849 small molecules. ChEMBL (https://www.ebi.ac.uk/chembl/) is a manually curated database for drug-like ligands and their activities (Gaulton et al., 2012). Its latest version 17 contains comprehensive information of more than 9000 targets and 1 million compounds derived from the literature.

*Determination of common off-targets*. The putative off-targets for each analgesic drug were determined by removing the known therapeutic targets from the corresponding non-redundant docking protein list. The combinatorial analgesic drug liquicet was analyzed by its two components, acetaminophen and hydrocodone, separately. The common off-targets for a designated SADR were determined by seeking the overlapped off-targets of analgesics according to their commonality in induction of the SADR.

*Identification of ADR-associated pathways*. The ADR-associated proteins (ADRAPs) are proteins that likely mediate ADRs via their binding to drugs or xenobiotics. The literature-reported ADRAPs were derived from the Drug-Induced Toxicity Related Proteins Database (DITOP, http://bioinf.xmu.edu.cn/databases/DITOP/index.html) (Zhang et al., 2007) and the literature. The DITOP database currently contains 618 distinct literature-reported ADRAPs, 529 drugs/ligands, and 418 distinct toxicity terms. The ADR-associated pathways are a group of biological pathways, dysfunction of which may directly induce ADRs. In this study, the ADR-associated pathways were obtained by mapping the known ADRAPs into the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (http://www.genome.jp/kegg/). Current version of KEGG database provides a collection of 449 pathway maps representing knowledge on molecular interaction and reaction networks (Kanehisa et al., 2012).

All ADR–pathway associations obtained for the six analgesics were integrated in a form of interaction network using the freeware Cytoscape with the yFiles Organic Layout algorithm (Shannon et al., 2003). For a