Co-administration of Atorvastatin Blocks CYP3A4: Exacerbated Risk of Interstitial Lung Disease

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INTRODUCTION

Interactions between two different drugs consumed simultaneously often lead to complications known as adverse drug events (ADEs), or unintended consequences of prescribed medication, which are often detrimental to patients' health (Zhan et al., 2018; Kovačević et al., 2019). Although it is reported that half of ADEs to be preventable, outcomes of drug-drug interactions (DDIs) are hard to predict *in vivo* and modern drug testing is typically done in standardized populations, which excludes the possible occurrence of ADEs (Venkataramanan et al., 2006; Wijnen et al., 2010; Hernández & Cruz-Gonzalez, 2018). Particularly, DDIs involving statins are of importance over 221 million statin prescriptions are dispensed to over 40 million individuals annually (Schwaiblmair, 2012; Olson et al., 2018). Statins are also frequently co-administered with other drugs, which may ultimately cause harmful DDIs (Genetic and Rare Diseases Information Center, 2019; Fig. 1).

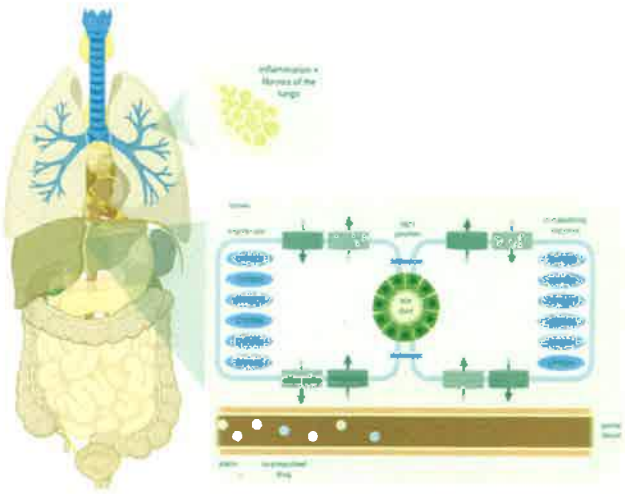


Fig. 1. Drug metabolism of statins through CYP enzymatic activity. Typically, statins are metabolized by CYP enzymes, however the co-administration of statins can lead to enzyme competition.

Past bioinformatics and systematic studies have suggested a possible ADE of statin administration to be interstitial lung disease (ILD), a progressive disease that causes an irreversible fibrosis of the lungs and possess five-year death rates as high as 80% (Saad et al., 2013). Current treatments for ILD are only capable of slowing the lung damage of the progressive disease (Kalchiem-Dekel et al., 2018). The lack of knowledge in the mechanism behind ILD contributes to the difficulty of predicting and understanding ILD as an ADE after the consumption of certain medication (Nasri et al., 2015). Moreover, the previous studies have

reached contradicting and dubious results between the association between statins and ILD, as some support the association, and others negate it (Fernandez et al., 2008; Saad et al., 2013; Zanger & Schwab, 2013; Lalic et al., 2016; Ramkumar et al., 2016). These existing contradictions in literature may be due to the fact that past studies have not taken into consideration drug-metabolizing enzymes, Cytochrome P450 (CYP), in statin-associated DDIs.

Pharmacokinetics and pharmacodynamics of drugs are determined by a delicate interplay among influx and efflux transporters, and drug-metabolizing enzymes (e.g., CYP) (Zhang et al., 2006; Kalliokoski & Niemi, 2009). For example, solute carrier organic anion transporters, such as SLCO1B1, SLCO1B3 and SLCO2B1, are mainly expressed in hepatocytes responsible for the uptake of endogenous compounds, xenobiotics and drugs from blood into hepatocytes (Niemi, 2007; Kalliokoski & Niemi, 2009). The influx transporters deliver their substrates to metabolizing enzymes (Nies et al., 2008), representing an important step toward elimination of drugs by metabolism or biliary excretion (Niemi 2007). On the other hand, ABC transporters, such as ABCB1, act as ATP-dependent efflux pumps for chemically diverse substances from the cells, decreasing intracellular concentrations of the substances (Bosquillon, 2010). Influx and efflux transporters and drug metabolizing enzymes are also expressed in the lung, responsible for detoxification of inhaled xenobiotics (Hamilton et al., 2002; Berg et al., 2014).

CYP enzymes are responsible for the majority of drug metabolism in the body and also serve as target enzymes of all statins -- atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin (Lee et al., 2016). Different CYP enzymes have different substrate profiles of statins (Licata et al., 2018). After consumption, statins are metabolized by a CYP enzyme through an oxidation process into its respective metabolites (Fong, 2016). The metabolite of the statin is then able to inhibit 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA)

reductase, which hinders cholesterol production (Jiang et al., 2018; Mohammadkhani et al., 2019). However, when statins are consumed simultaneously with another drug that shares a common target CYP enzyme, both drugs compete for the CYP enzyme. As a result, the majority of statins may not be metabolized by a CYP enzyme and fail to perform their designated function (lowering cholesterol levels) (Zhelyazkova-Savova et al., 2014). The concentration of the pre-metabolized form of the statin in the plasma of the blood remains relatively high, which increases the likelihood of the occurrence of an ADE (Ward et al., 2019). Statins are also substrates of influx transporters, such as SLCO2B1 (Kopplow et al., 2005; Satoh et al., 2005; Grube et al., 2006; Kalliokoski & Niemi, 2009). Notably, atorvastatin, fluvastatin, pitavastatin, rosuvastatin can inhibit SLCO2B1 as well (Karlgren et al., 2012) in a concentration-dependent manner (Kashihara et al., 2017). Atorvastatin is also a substrate as well as an inhibitor of ABCB1 (Wu et al., 2000; Boyd et al., 2000; Rodrigues et al., 2006; Rodrigues et al., 2009; Guan et al., 2019). Interference with the uptake and excretion by transporters by statins together with inhibition of CYP enzymes by co-administered drugs complicates the assessment of each contribution in the pharmacokinetic profile of drugs and cause unpredictable drug-drug interactions (Benet et al., 2003; Benet et al., 2004; Ho & Kim, 2005; Zhang et al., 2006; Poirier et al., 2007; Kalliokoski & Niemi, 2009; Kashihara et al., 2017). Active transport mechanisms (both influx and efflux) between different parts of the body, such as the respiratory tract and the gastrointestinal tract, can influence each other on the biodistribution of drugs and xenobiotics (Ayrton et al., 2001), providing additional complexity. In the early 2000's almost half of drugs were withdrawn from the US market due to unexpected DDIs (Zhang et al., 2006), further confirming our limited capability of DDI prediction.

The overall objective of this study is to elucidate the prognosis of ILD through the co-administration of statins with CYP-inhibiting drugs by computing statistical associations between the administration and co-administration of statins and the occurrence of ILD, identifying natural compounds with unknown CYP inhibition with increased risk of ILD when taken with statins, and revealing biological pathways potentially associated with ILD when co-administered with statins.

METHODS

PRR calculations of individual statins

The proportional reporting ratios (PRRs) of the individual statins for ILD were calculated using the propensity score matching protocol listed in *Data-Driven Prediction of Drug Effects and Interactions* using the FDA Adverse Events Reporting System (FAERS) database (Tatonetti et al., 2013). PRRs for ILD of other single compounds were computed similarly.

Extracting combinations of statin and drugs from FAERS dataset

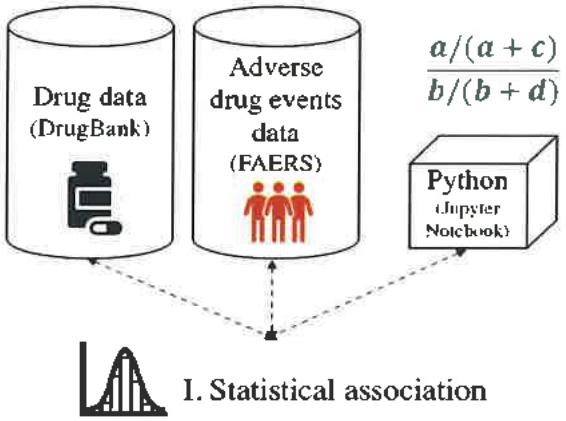
Each individual statin was annotated in terms of its metabolizing CYP enzymes using DrugBank 5.1.4. The CYP enzymes were then annotated in terms of drug inhibitors and the drug inhibitors were annotated in terms of relative inhibition strength (unknown, weak, moderate, strong) and drug purpose/target. All annotations were stored in a matrix in a comma-separated values (CSV) file. Two separate sets of combinations of statin and drug were created using the annotations. For a given inhibitor, one set contained statin and drug pairs that shared a common CYP enzyme target, whereas the other set consisted of statin and drug pairs that did not share a common CYP enzyme target. For both sets of statin and drug pairs, cases from the FAERS database where the particular statin and inhibitor were consumed and resulted in the ADE of ILD

were extracted. From the extracted files, the following data values were obtained: the number of cases where the patients a) consumed the particular statin and inhibitor pair and was diagnosed with ILD as a result, b) consumed the particular statin and inhibitor pair and was not diagnosed with ILD as a result, c) did not take the particular statin and inhibitor pair and was diagnosed with ILD as a result, and d) did not take the particular statin and inhibitor pair and was not diagnosed with ILD as a result.

PRR calculations

The PRRs for each statin and inhibitor pair were calculated. The distributions of PRRs were graphed with a histogram and a stats.gamma fit on density plots using the Python package Seaborn 0.9.0 on Jupyter Notebook on Anaconda Navigator [24]. The PRRs were then converted into log(PRRs) by taking the logarithm of the PRRs in base 10. The log(PRRs) that resulted in undefined numbers (i.e., taking the logarithm of zero) were excluded from this dataset. The distributions for the log(PRRs) were graphed with a histogram and a stats.gamma fit on density plots using the Python package Seaborn 0.9.0 on Jupyter Notebook on Anaconda Navigator (Anaconda, Inc). Significance tests were performed using a 2-sample z-test at an $\alpha = 0.05$ level.

PRRs from the sets extracted earlier were calculated for the ADE of lung cancer as described above. A 2-sample z-test was used to test significance.



Administration

	ILD	all other ADEs
statin	a	c
all other drugs	b	d

Co-administration

	ILD	all other ADEs
statin + inhibitor	a	c
all other drug combinations	b	d

Fig. 2. Methodology of determining statistical associations between ILD and administration of statins (or co-administration of statin + CYP inhibiting drug) using PRRs. The CYP inhibitors selected for calculation target for CYP variants that metabolize statin.

Molecular docking

Using the ILD cases from FAERS data that had PRRs greater than 1, natural products with a chemical similarity to the original drug were retried from the ZINC library (Irwin and Shoichet, 2005). The HEM ligand was manually removed from the CYP3A4 to allow molecular docking to enzyme. A natural product was computationally docked to CYP3A4 using the software SwissDock (Grosdidier et al., 2011). Bindings of compounds to CYP3A4 were visualized using USCF Chimera.

Construction of biological networks

Off-targets of statins expressed in the lung were recorded using ChemBL and key proteins found in the surfactant metabolism were determined using Reactome. BioGRID, Proteomics DB, String, and Corum were applied to determine interactions associated with off-targets and proteins in the surfactant metabolism. The database Gene was applied to measure the gene expression in the lung. All interactions were then visualized using Cytoscape 3.7.0.

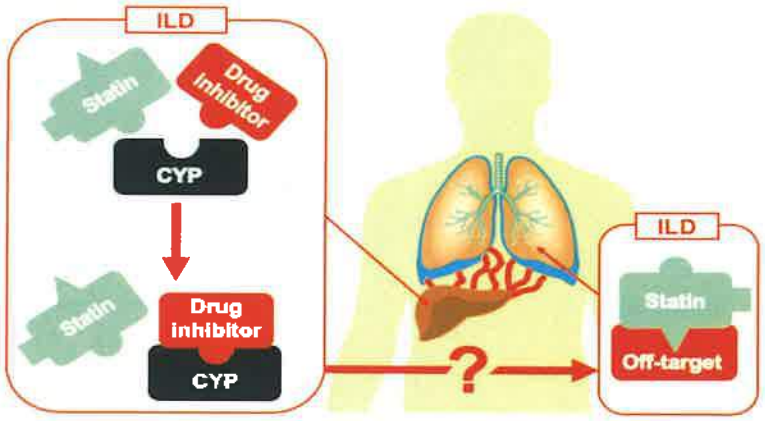


Fig. 6. Proposed mechanism of ILD by co-administration of atorvastatin and CYP3A4 inhibitor. Limited metabolization of atorvastatin by CYP3A4 due to a drug inhibitor may cause its binding to an off-target highly expressed in the lung to cause ILD.

Proof of concept: Myopathy caused by the co-administration of atorvastatin with CYP drug inhibitors

Using the previously extracted files and values from the FAERS database, PRRs were calculated using the formula $\frac{a/(a+c)}{b/(b+d)}$ with Python 3.6.5 for the ADE of myopathy. The PRRs were grouped based upon common CYP interaction using DrugBank XXXX. Based on literature review, the PRRs of the combinations of statin and CYP drug inhibitor were grouped upon the CYP interactions: CYP2D6, CYP3A4, and CYP3A5. The PRRs were converted into log(PRR)s in base 10 and the distributions of log(PRR)s for the combinations of statin and CYP drug inhibitor that share and do not share a common CYP interaction were graphed with a histogram and stats.gamma fit on density plots using Seaborn 0.9.0, Pandas 0.25.3, SciPy 1.3.3, and Matplotlib 3.1.1. The top six drugs co-administered with a statin with the highest PRRs were

recorded with their corresponding PRR and the association of the co-administered statin and CYP inhibitor drug pair to an increased risk of myopathy were confirmed via literature review. In order to create a biological pathway behind the onset of myopathy, off-targets of statins were recorded from ChEMBL and tissue-specific protein expression will be determined from NCBI Gene 234.0 data. Protein-protein interactions were examined between the recorded proteins using Reactome 70, BioGRID 3.5, Proteomics DB 3.0, STRING 11.0, and CORUM 3.0 and visualized with Cytoscape 3.7.0. The protein-protein interaction found to be underlying the onset of myopathy caused by the co-administration of statin and CYP drug inhibitors were supported with a literature review.

RESULTS AND DISCUSSION

PRRs of individual statins

PRRs of individual statins for ADE of ILD were computationally calculated using FAERS. For ILD, the PRRs of lovastatin and simvastatin were less than 1, the PRR of atorvastatin was approximately 1 (PRR = 1.01), and the PRRs of rosuvastatin, pravastatin, fluvastatin, and pitavastatin were greater than 1 (Table 1).

Table. 1. PRR scores of the seven FDA-approved statins for the ADE of ILD. PRR scores were calculated with Python on Anaconda Navigator using FAERS data. The PRR score of atorvastatin was found to be 1.01, or statistically neutral.

Statin	PRR
Lovastatin	0.36
Simvastatin	0.90
Atorvastatin	1.01
Rosuvastatin	1.22
Pravastatin	1.26
Fluvastatin	1.96
Pitavastatin	2.11

Because the PRR of atorvastatin is approximately 1 (Table 1), there is a statistically neutral association between the consumption of atorvastatin and the resulting ADE of ILD. This suggests that ILD does not result as an ADE due to the consumption of atorvastatin alone, although the onset of

ILD due to the co-administration of atorvastatin with another drug can't be ruled out.

In order to determine if the DDIs caused by the co-administration of statins and a CYP drug inhibitor is associated with ILD, rather than the administration of the CYP drug inhibitor itself, the PRRs of the combinations of atorvastatin and CYP drug inhibitor for ILD were plotted against the PRRs of CYP drug inhibitors themselves for ILD in a scatter plot. There was a significant difference in the number of PRRs greater than one for the co-administration of atorvastatin and CYP drug inhibitor than the number of PRRs greater than one for the single administration of CYP drug inhibitors (Fig. 3).

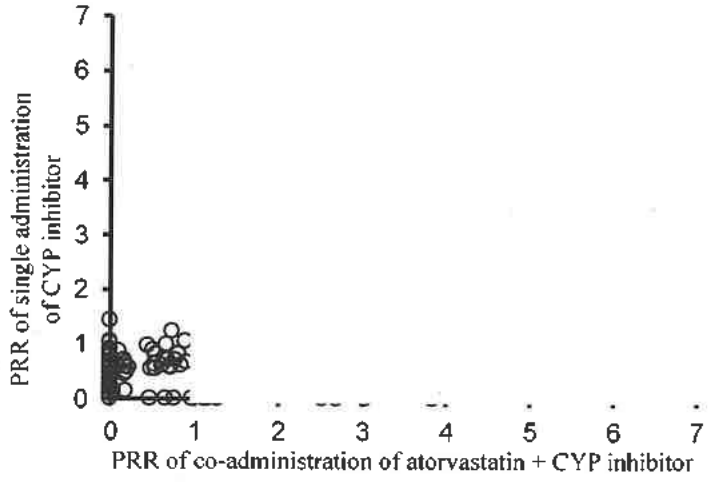


Fig. 3. Correlation between PRRs for co-administration of atorvastatin + CYP inhibitor vs. single administration of CYP inhibitor. Red- vs. blue-shaded areas: Onset of ILD occurs due to drug-drug interactions between atorvastatin and CYP inhibitor.

The significant difference between the number of PRRs greater than one for the co-administration of atorvastatin with a CYP drug inhibitor and the number of PRRs greater than one for the single administration of a CYP drug inhibitor inducates that most cases of ILD is not due to the single administration of atorvastatin or the CYP drug inhibitor, but instead due to DDIs occurring between atorvastatin and the CYP drug inhibitor.

Combinations of atorvastatin and drugs retried from FAERS dataset, and their PRR calculations for ILD

From the annotated statins, CYP enzymes, and drug inhibitors, 1,990 statin and drug combinations that share a common target CYP enzyme and 1,132 statin and drug combinations that do not were produced from FAERS datasets. Among different statins, co-administration cases of atorvastatin and other drug were extracted from FAERS. Seventy-nine atorvastatin and drug combinations that share a common target CYP enzyme and 150 statin and drug combinations that do not were filtered from FAERS.

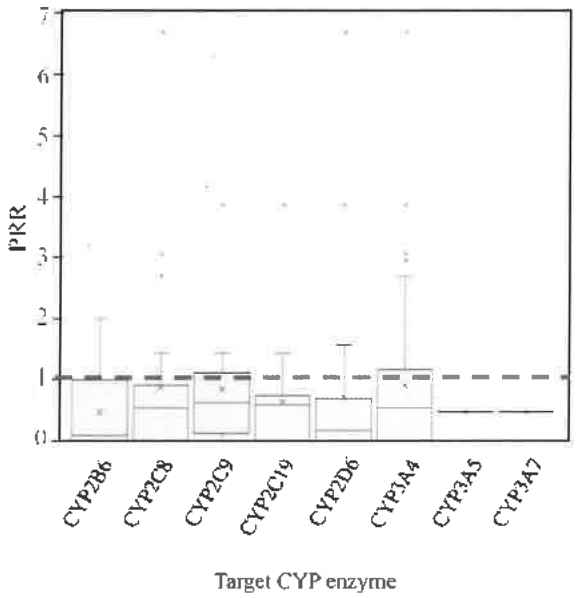


Fig. 2. Distributions of PRR scores of atorvastatin and drug combinations that share a common target CYP enzyme grouped by common CYP interaction. PRR scores were calculated with Python on Anaconda Navigator using FAERS data. CYP3A4 had the greatest proportion and number of cases with PRR scores over 1.

The PRRs of the extracted atorvastatin and drug combinations were then computed and analyzed in groups of common CYP interaction. Combinations of atorvastatin and a CYP3A4-inhibiting drug showed the greatest proportion and number of combinations with PRRs greater than 1 for ILD (Fig. 2).

The finding that the co-administration of atorvastatin and another drug resulted in ILD in FAERS (Fig. 2) supports that ILD was not a result of the single administration of atorvastatin, but the co-administration of atorvastatin with another drug. Specifically, the PRR calculations indicate that drugs inhibiting CYP3A4 are more likely to cause ILD with consumption with atorvastatin, suggesting the potential importance of CYP3A4 in statin DDIs.

The distributions of the log(PRRs) of the atorvastatin and drug combinations were then graphed on density plots with a stats.gamma fit using Python. The means of the log (PRRs) distribution for the atorvastatin and drug combination that share a common target CYP enzyme was not statistically different from the means for the combination not sharing a target CYP enzyme, when all relevant CYP enzymes were considered (Fig 3). In contrast, a significant difference ($p=0.031$) was found when the comparison was restricted to CYP3A4 as a common target enzyme.

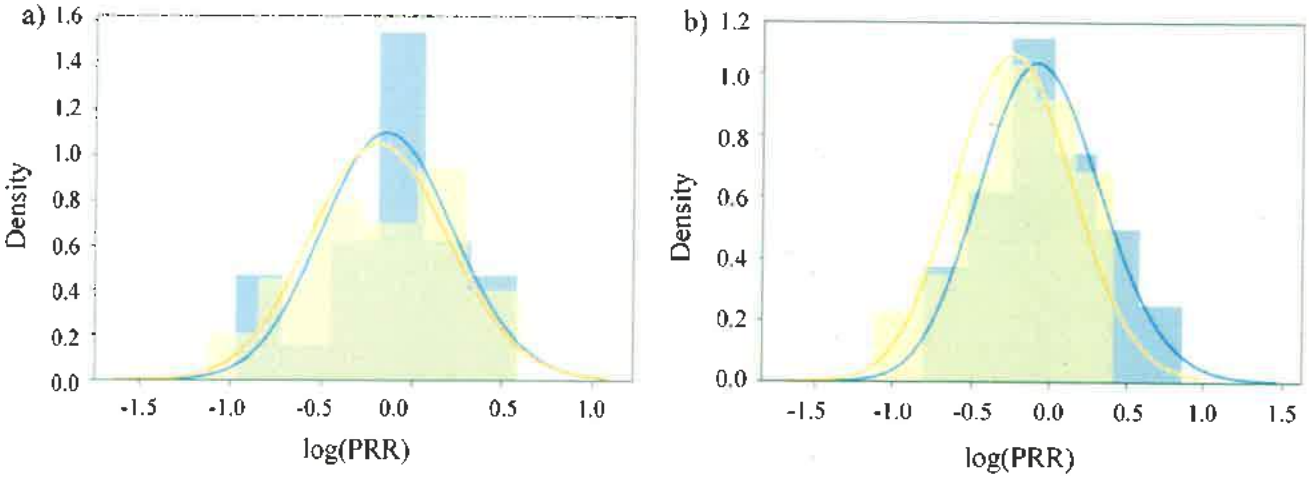


Fig. 3. Distributions of log(PRR) scores of non-metabolizable atorvastatin + other drugs (blue), metabolizable atorvastatin + other drugs (orange) by a) all relevant CYP variants and b) CYP3A4 only on density plots. Density plots were graphed using the Python package Seaborn 0.9.0. Distributions for all relevant CYP variants showed no significant difference. Distributions for only CYP3A4 showed a significant difference; $p = 0.031$. Significant tests were performed using a 2-sample z-test.

The statistical analysis on combinations of atorvastatin and other drugs (Fig. 3) further supports the key role of CYP3A4 in the statin-induced ILD. CYP3A4 plays a critical role in metabolism of drugs and other endogenous and exogenous compounds, responsible for detoxification of xenobiotics. Drugs that can increase plasma concentrations of statins by inhibiting CYP3A4 can cause adverse effects, such as ILD (Fig. 3), as is the case with combinations of statins and drugs leading to skeletal muscle toxicity (Rendic & Carlo, 1997).

Thus, the co-administration of atorvastatin with CYP3A4 drug inhibitors should be avoided to prevent ILD as an ADE in patients.

Molecular docking of natural compounds for a potential risk of ILD

Motivated by the aforementioned result that CYP3A4 inhibitors can cause ILD when co-administered with atorvastatin, natural products not reported in FAERS but possibly implicated in ILD were examined. Among different natural products curated in the ZINC library (Irwin & Shoichet, 2005), diphenhydramine was chosen because its high structural similarity (similarity score: 0.71) with orphenadrine having a PRR = 2 when co-administered with atorvastatin. The molecular docking of diphenhydramine to CYP3A4 was subsequently conducted using SwissDock (Grosdidier et al., 2011). A similar docking was also performed with atorvastatin and orphenadrine. The molecular dockings of CYP3A4 with atorvastatin, orphenadrine, and diphenhydramine showed a similar binding to CYP3A4 by all three compounds (Fig. 4).

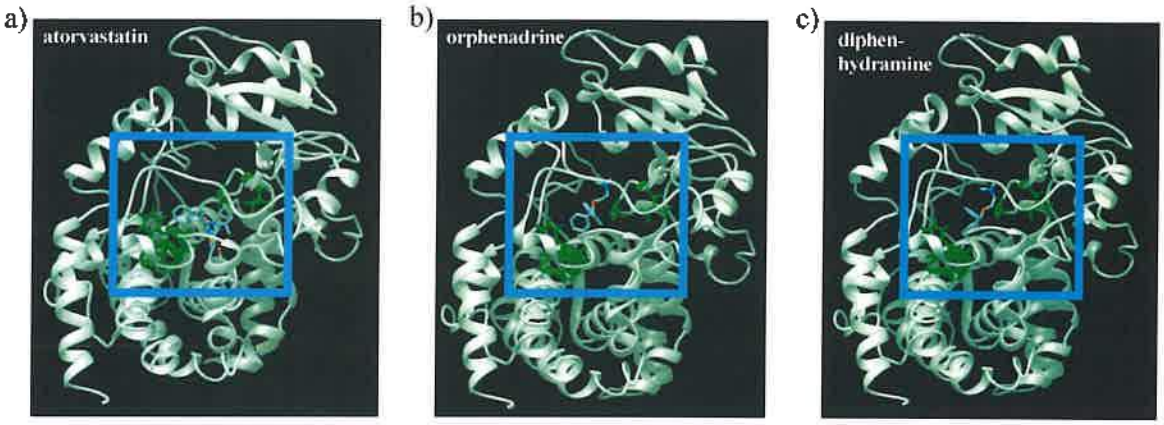


Fig. 4. Molecular docking of a) atorvastatin, b) orphenadrine, and c) diphenhydramine with CYP3A4. Molecular docking was performed using chemical data from ChemBL and ZINC through the software SwissDock. Atorvastatin, orphenadrine (PRR for ILD = 2 when co-administered with atorvastatin), and a natural product (diphenhydramine) with high chemical similarities to orphenadrine bind similarly to CYP3A4. The green visualization represents binding site of CYP3A4, which is surrounded by a blue box. HEM not shown.

The molecular docking results supports that atorvastatin (Fig. 4a), orphenadrine (Fig. 4b) and its chemically similar natural product, diphenhydramine (Fig. 4c), all bind to the active site of CYP3A4. While orphenadrine was found to inhibit CYP3A4 (Coultas et al., 1994; Herazo-

Maya et al., 2017), it is currently unknown whether diphenhydramine is also an inhibitor of CYP3A4 or just a substrate of this enzyme. CYP3A4's extreme promiscuity in substrate specificity (Hayes et al., 2014) imply that a large number of relatively unknown compounds may be identified as an inhibitor or substrate in the near future.

Off-targets of atorvastatin in the lung

Given a consideration of an enhanced plasma concentration of atorvastatin upon inhibition of CYP3A4 by co-administered drugs, potential off-targets of atorvastatin were examined from the ChemBL database. The two off-targets of atorvastatin significantly expressed in the lung were identified to be an organic anionic influx transporter, SLCO2B1, and an ABC efflux transporter, ABCB1.

To further verify the implication of the two off-targets in ILD, the PRRs of single compounds reported in FAERS for ILD and the fractions of off-target binders in the two groups of PRRs > or ≤ 1 were calculated. Five out of 321 single compounds with PRRs greater than 1 was reported to bind to ABCB1, in contrast to 16 out of 1,562 single compounds for PRRs less than 1. On the other hand, fifty out of 321 single compounds with PRRs greater than 1 was found to bind to SLCO2B1, which is compared with 168 out of 1,562 single compounds for PRRs less. The fraction of SLCO2B1 binders is significantly greater among compounds with PRR > 1 when compared to those with PRR ≤ 1, demonstrating the direct relevance of SLCO2B1 in ILD. A similar trend was observed for ABCB1 binders, though not statistically significant due to their relatively small numbers.

A bioinformatic survey on off-target proteins for atorvastatin suggest the involvement of two transporter proteins, ABCB1 and SLCO2B1, in the pathology of ILD caused by co-

administration of atorvastatin and other drugs. ABCB1 is usually found on the apical surface of epithelial cells, eliminating substrates from systemic circular through biliary excretion (Hamilton et al., 2002) and thus protecting tissues from toxicity of xenobiotics (Hamilton et al., 2002). ABCB1 can be associated with ILD via β-catenin. Dysregulation of β-catenin signaling causes pulmonary fibrosis, a subtype of ILD, and downregulates ABCB1 (Katoh, 2018). Notably, there is no statistically significant association between ABCB1 single nucleotide polymorphisms and idiopathic pulmonary fibrosis (IPF), a subset of ILD (Martinelli et al., 2015). It should also be noted that inhibitory effects of various statins for ABCB1 were reported in the following order: simvastatin (strongest), lovastatin, atorvastatin and pravastatin (weakest) according to IC_{50} (Yang et al., 1996). The trend is generally opposite to PRR scores of statins alone for ILD (Table 1). Taken together, the implication is that inhibiting ABCB1 alone may not be sufficient to cause the statin-associated ILD, demonstrating the importance of another major off-target, SLCO2B1. SLCO2B1 transports a wide range of endogenous and exogenous molecules, such as estrone-3-sulfate and other xenobiotics (Want et al., 2001).

Atorvastatin, metabolizable by CYP3A4, is a substrate and an inhibitor of ABCB1 and SLCO2B1 (Kopplow et al., 2005; Satoh et al., 2005; Grube et al., 2006; Kalliokoski & Niemi, 2009; Karlgren et al., 2012). The inhibitory mechanisms of ABCB1 and SLCO2B1 by atorvastatin can interfere with the uptake of atorvastatin from blood to the liver cells and its elimination from systemic circulation (Mealey 2004; Kashihara et al., 2017). Thus, the interferences together with the limited metabolism of atorvastatin by CYP3A4 due to co-administered drugs can greatly enhance systemic exposure of atorvastatin (Rodrigues et al., 2009) and eventually result in an increased accumulation of atorvastatin in the lung (van der Deen et al., 2005).

ABCB1, SLCO2B1 and non-CYP3A4-drug metabolizing enzymes are also located in the lung, contributing to the absorption, distribution, metabolism and elimination of inhaled xenobiotics in the pulmonary tissue (Hamilton et al., 2002; Bosquillon et al., 2010; Berg et al., 2014). Thus, inhibition of SLCO2B1 by atorvastatin might cause ILD by lowering the absorption of inhaled drug administered for the treatment of lung diseases. The airways (trachea, bronchi, bronchioles and terminal bronchioles) of the lung are continuously to pathogens and other toxic agents, such as cigarette smoke components and those producing oxidative stress (van der Deen et al., 2005). ABCB1 may protect against oxidative stress and toxic compounds generated upon cigarette smoking as well as other inhaled toxic substances by acting as cellular efflux pumps (van der Deen et al., 2006). Thus, inhibition of ABCB1 can increase the risk of ILD caused by smoking and inhalation of dust containing silica and asbestos fibers (Charlesworth Press, 2013).

Biological networks around off-targets of atorvastatin implicated in ILD

To identify a set of other biological molecules in the lung potentially implicated in ILD caused by co-administration of atorvastatin and other drugs, biological pathways around the two off-targets of atorvastatin, SLCO2B1 and ABCB1, were created (Fig. 5). In the pathways, SLCO2B1 and ABCB1 were connected to the biological components implicated in one of major ILD mechanisms - dysregulation of surfactant production, metabolism and homeostasis. The connections were made through direct interacting partners of SLCO2B1, ABCB1 and ILD-associated components, all identified from comprehensive bioinformatic database and tools.

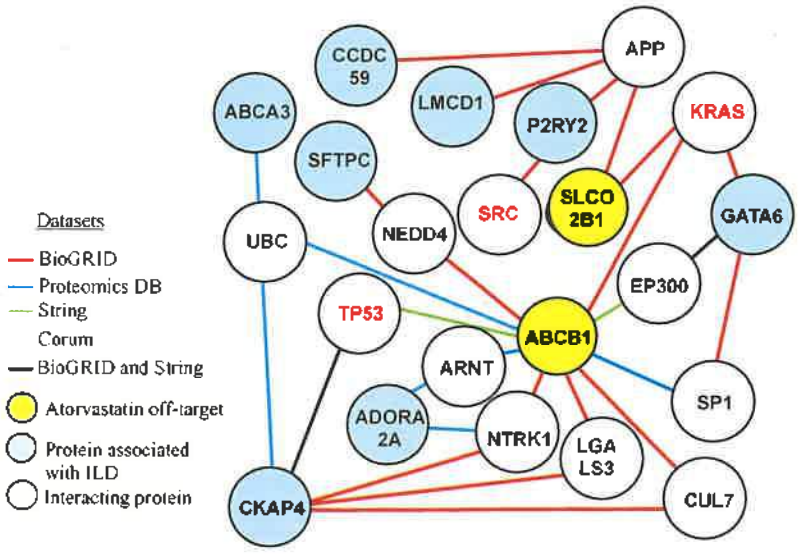


Fig. 7. Protein interactions in the lung implicated in ILD caused by co-administration of atorvastatin + CYP3A4 inhibitor. Binding of atorvastatin to ABCB1 or SLCO2B1 may interfere the network of desired interactions and cause ILD.

The PRRs of the atorvastatin and drug combinations that had PRRs greater than 1 for ILD were calculated for the ADE of lung cancer using FAERS. None of combinations of atorvastatin and other drugs having PRR scores greater than 1 for ILD showed PRR scores greater than 1 for lung cancer. The implication is that oncogenes or tumor proteins are less likely to be involved in the statin-induced ILD.

When oncogenes or tumor proteins are excluded from further consideration, a linkage of ABCB1 to surfactant protein C, SFTPC, via an E3 ubiquitin ligase, NEDD4, was identified (Fig. 5). NEDD4 regulates the ubiquitination and proteasome-dependent degradation of a number of proteins. ABCB1 is a substrate of NEDD4, and its quantity is negatively correlated with NEDD4 activity (Akkaya et al., 2015). NEDDR is required to produce SFTPC from SFTPC's precursor at the alveolar type II epithelial cells (Kotorashvili et al., 2009). SFTPC plays a key role in lowering surface tension at the air-liquid interface in alveoli and maintaining the structural integrity of alveoli (Agassandian & Mallampalli 2013). Dysregulation of SFTPC can cause ILD (Whitsett et al., 2010; Akella & Deshpande, 2013).

ABCB1 can also be connected to GATA6 via SP1 (Fig. 5). Expression of the *ABCB1* gene can be controlled by a transcription factor, SP1 (Gromnicova et al., 2012), which is found to interact with another transcription factor, GATA6 (Zhou et al., 2008; Flodby et al., 2017).

GATA6 controls transcription of surfactant protein A (SFTPA) genes at the air-liquid interface in the alveoli (Bruno et al. 2000). Another ABC transporter, ABCA3 (Fig. 5), is directly involved in surfactant production and regulation in the alveolar type II cells in the lung (Whitsett et al., 2015), though no study has yet to be conducted for binding of atorvastatin to ABCA3.

No significant functional connection was found around SLCO2B1 from literature, indicating either not every pathway shown is biologically active or additional studies needs for its verification (Fig. 5). However, according to the proposed biological network for the ILD induced by co-administration of atorvastatin and CYP3A4-inhibiting drugs (Fig. 5), APP can directly bind to three proteins important in ILD pathology, CCDC59, LMCD1 and P2RY2 (Fig. 5). While APP is believed to be primarily associated with Alzheimer's disease (Nozawa et al., 2002), APP was also found to be directly associated with HMGB1, a key upstream mediator in chronic airway diseases, such as chronic obstructive pulmonary disease (O'Brien & Wong, 2011; Wong et al., 2017). A further study is required to verify a biological connection between SLCO2B1 and APP in ILD pathology.

Proof of concept: Myopathy caused by the co-administration of atorvastatin with CYP drug inhibitors

The top six drugs with the greatest PRRs for myopathy when co-administered with a statin were found to be: probenecid (PRR=30), miconazole (PRR=20), clotrimazole (PRR=16.4), ezetimibe (PRR=12.1), candesartan (PRR=10.4), and fenofibrate (PRR=10.1; Table2). All compounds found to be statistically associated with myopathy when co-administered with a statin were supported with a literature review (Table 2).

Table 2. Compounds co-administered with statin having top 6 PRRs for myopathy

Drug co-administered with statin	PRR	Literature Support
Probenecid	30	Yes [28]
Miconazole	20	Yes [28]
Clotrimazole	16.4	Yes [28]
Ezetimibe	12.1	Yes [28]
Candesartan	10.4	Yes [28]
Fenofibrate	10.1	Yes [28]

The support from literature regarding the statistical associations found between the co-administration of statin and CYP drug inhibitors for the ADE of myopathy support the statistical associations previously found between the co-administration of atorvastatin and CYP drug inhibitors for the ADE of ILD. Thus, this supports that the co-administration of atorvastatin with a CYP3A4 drug inhibitor is responsible for the secondary onset of ILD in patients.

Through literature review, it was found that CYP2D6, CYP3A4, and CYP3A4 were key CYP enzymes responsible in the onset of statin-induced myopathy. A significant difference was found between the distributions of log(PRR)s for: statin and CYP drug inhibitors that share a common CYP interaction and statin and CYP drug inhibitors that do not share a common CYP interaction ($p<0.01$; Fig. 8).

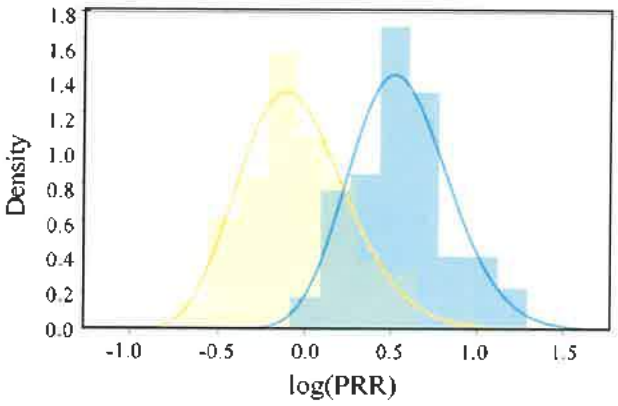


Fig. 8. log(PRR)s of (blue) statin + CYP2D6, CYP3A4 or CYP3A5 inhibitor and (red) statin + other CYP inhibitor. CYP2D6, CYP3A4 and CYP3A5 are known to be associated with statin-induced myopathy ($p < 0.01$)

The confirmation of the significant difference between the two distributions (Fig. 8) of statin and CYP drug inhibitors grouped based on common CYP interaction (CYP2D6, CYP3A4, CYP3A5) for the ADE of myopathy support the previous finding that showed a significant difference between the two distributions of statin and CYP drug inhibitors grouped based on common CYP interaction (CYP3A4) for the ADE of ILD. Thus, the co-administration of atorvastatin and CYP3A4 inhibitors are responsible for the secondary onset of ILD.

To identify a biological mechanism of the onset of myopathy induced by the co-administration of statins with CYP drug inhibitors, a biological pathway around the identified off-target of HMGCR were created (Fig. 9). The interactions among the different proteins were identified from comprehensive bioinformatics databases and tools (Fig. 9).

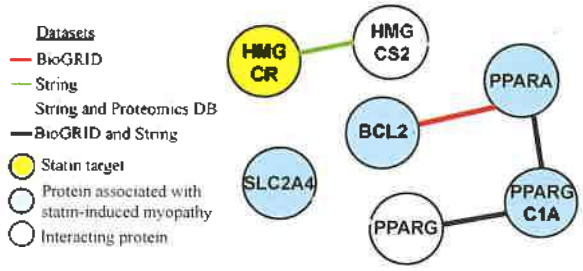


Fig. 9. Protein/gene interactions implicated in statin-induced myopathy. Binding of statin to HMGCR may interfere with the protein network of desired interactions and result in myopathy.

Due to successful enzyme competition and thus, an increased concentration of the statin in the plasma of the blood, the statin may bind to one of its off-targets. The binding of the statin to HMG-CoA reductase (HMGCR) may interfere with a series of subsequent protein interactions in myocytes and induce myopathy in patients (Fig. 9). The role of direct binding to HMGCR of the statin in the onset of myopathy caused by the co-administration of statin and CYP drug inhibitor was supported by literature review. Thus, the biological network and mechanism found behind the onset of ILD caused by the co-administration of atorvastatin with a CYP3A4 inhibitor is likely the biological mechanism behind the ADE.

CONCLUSION

This study demonstrates a statistically significant association between the co-administration of atorvastatin and other drugs that both interact with CYP3A4 and the risk of ILD, indicating CYP3A4 plays a direct role in inducing ILD.

Physicians should avoid simultaneously prescribing atorvastatin with other drugs, which is highly common today, to avoid chances of the patient acquiring ILD as an ADE, specifically those interacting or inhibiting CYP3A4 (LINCS L1000FWD; Wang et al., 2018). By doing so,

the onset of ILD as an ADE can be reduced (Gates et al., 2018). A novel pathological mechanism behind ILD was revealed using the off-targets of atorvastatin highly expressed in the lung, SLCO2B1 and ABCB1 (Fig. 6), which will be useful for a deep understanding of ILD as an ADE on the pathway level (Fujiwara et al., 2016). Understanding key protein-protein and other biological interactions through this mechanism can ultimately give way to the development of general treatments for ILD in the future (Greiffo et al., 2017). Diphenhydramine was indicated as a natural product that could cause ILD in patients following its co-administration with atorvastatin due to its similar binding to CYP3A4 (Iwase et al., 2017). Although diphenhydramine is not reported in FAERS database, the natural product should not be prescribed simultaneously with atorvastatin to patients due to its likelihood of causing ILD.

Future work in this study includes an examination of whether demographic differences of individuals affect susceptibility of the statin-induced ILD, motivated by a previous report that the prevalence of ILD was 20% higher in males (Coultas et al., 1994). The examination will require more extensive sets of FAERS data, gender- and ethnic origin-dependent protein expression profiles. The clinical difference is also worthy of investigation, as some medical treatments, such as immunosuppression use, were found more frequently in high-risk groups for ILD (Herazo-Maya et al., 2017). In another future work, the proposed biological pathway for statin-induced ILD may be further fine-tuned through similar statistical analyses of FAERS database by calculating PRRs of drug combinations for other diseases related to ILD, such as Chronic Obstructive Pulmonary Disease (COPD) (Hanlon et al., 2018). In this future study, a correlation between disease similarity and the number of overlapping drug combinations for high PPR values can be explored. In addition, the present study can be extended to an ILD mechanism

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