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Identification and Mechanistic Investigation of Drug-Drug Interactions Associated with Myopathy – A Translational Approach

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Abstract

Myopathy is a group of muscle diseases that can be induced or exacerbated by drug-drug interactions (DDIs). We sought to identify clinically important myopathic DDIs and elucidate their underlying mechanisms. Five DDIs were found to increase the risk of myopathy based on analysis of observational data from the Indiana Network of Patient Care. Loratadine interacted with simvastatin (relative risk 95% CI = [1.39, 2.06]), alprazolam (1.50, 2.31), ropinirole (2.06, 5.00) and omeprazole (1.15, 1.38). Promethazine interacted with tegaserod (1.94, 4.64). *In vitro* investigation showed that these DDIs were unlikely to result from inhibition of drug metabolism by CYP450 enzymes or from inhibition of hepatic uptake via the membrane transporter OATP1B1/1B3. However, we did observe *in vitro* synergistic myotoxicity of simvastatin and

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

X.H. designed and performed the experiments, generated data, and contributed to writing of the manuscript. S.K.Q. and Z.W. generated data and performed the statistical analyses. P.Z. and J.D. performed data analysis and D.A.F. provided pharmacology support. Z.D. and J.S.E. helped to perform the experiments. L.L. conceived the project and co-wrote the manuscript.

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desloratadine, suggesting a role in loratadine-simvastatin interaction. This interaction was epidemiologically confirmed (odds ratio 95% CI = [2.02, 3.65]) using the data from the FDA Adverse Event Reporting System.

Keywords

drug-drug interaction; myopathy; translational; pharmacoepidemiology; screening; CYP; OATP; synergic myotoxicity

INTRODUCTION

Drug-induced myopathy, among the most common causes of muscle disease (1), has clinical presentations ranging from asymptomatic muscle enzyme elevation to massive rhabdomyolysis with acute renal failure (2). Among 7 million case reports in the FDA Adverse Event Reporting System (FAERS) from 2001–2010, about 100,000 cases involved myopathy as suspected adverse drug reaction (ADR) (3). Among various drug classes associated with myopathy, statins have received extensive public and scientific attention. Statin-induced myopathy occurs in 5–20% of patients and is a significant barrier to maximizing the benefits of statin therapy (4). Considering that more than 18% of Americans aged 45 (approximately 127 million) took statins in 2012, 1.1 to 4.6 million patients might have experienced myopathy in 2012 alone.

Drug-induced myopathy can be exacerbated by pharmacokinetic and/or pharmacodynamic drug-drug interactions (DDIs). In a pharmacokinetic myopathic DDI, the object drug induces myopathy, and the precipitant drug modifies the object drug's myopathic effects by changing its pharmacokinetics. One such example is the interaction between cerivastatin and gemfibrozil that contributed to the withdrawal of cerivastatin from the market (5). The risk of cerivastatin-induced rhabdomyolysis is 10-fold higher than that of other statins; with concurrent use of gemfibrozil, a drug that substantially inhibits the metabolism of cerivastatin, the risk is 50-fold higher (6).

Although drug-induced myopathy and the role of DDIs as risk factors have been well documented, to our knowledge, no study has attempted to identify and investigate unknown myopathic DDIs systematically. Research on DDIs has been mostly limited to pharmacokinetic DDIs with identifiable mechanisms, a small scope, a relatively low efficiency and often a low clinical relevance. Recognizing the need for a translational approach for the study of DDIs (7), a promising new strategy involves pairing epidemiological studies with mechanistic investigations such as *in vitro* screening for metabolism-based DDIs. This approach was recently successfully applied to the study of interactions between sulfonylureas and statins/fibrates (8). Our previous study predicted 13,197 potentially interacting drug pairs using data mined from PubMed abstracts (9), and narrowed down to 3,670 clinically prescribed drug pairs using data derived from electronic medical records (9). In the current study, by applying a large-scale, translational approach, we sought to identify interacting drug pairs associated with myopathy and to elucidate their underlying pharmacokinetic and pharmacodynamic mechanisms.

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RESULTS

DDIs associated with increased risk of myopathy

We applied the myopathy concept definition (Supplementary Table S1) to a subset (n= 828,905) of the Indiana Network for Patient Care (INPC) database (2004–2009) formatted in the Observational Medical Outcomes Partnership (10) Common Data Model. We identified 59,572 myopathy cases, of which 48,877 (5.9%) had myalgia and myositis, 12,720 (1.5%) had muscle weakness, and 53 (0.0064%) had rhabdomyolysis. For each of the 3,670 drug pairs that we previously predicted to interact (9), we performed a simple cohort study. The demographics of the patient population were described previously (9) and are shown in Supplementary Table S2. Since race information was missing for 65.8% of the patients, it was not included in the analyses. For each drug pair, we estimated a risk ratio (RR) adjusted for age and sex, both known risk factors of myopathy (11). An RR greater than 1 indicated that the incidence of myopathy following the prescriptions for both drugs was greater than the additive incidence following a prescription for either drug alone. Drug pairs with RRs greater than 1 were therefore considered to be interacting and associated with an increased risk of myopathy. As a small sample size may yield an unreliable estimate of risk ratio, drug pairs with counts of myopathy cases less than 100 were excluded. We identified five DDIs associated with an increased risk of myopathy (Table 1), four of which involved the widely used antihistamine loratadine. The risk of myopathy increased with age at 1.0015 (95% CI = (1.00148, 1.00152)) per year, and was 1.64-fold (95% CI = (1.63, 1.65)) higher in females (8.6%) than in males (5.4%) (Supplementary Table S3). Since sicker patients tend to take more medications, we used the number of prescribed medications, including the relevant drug pair, within drug exposure windows to adjust for confounding by morbidity. The average number of prescribed medications was 3.8 ± 2.5. The five DDIs remained significant after adjusting for the number of co-prescribed medications (Supplementary Table S4).

Inhibition of CYP-mediated drug metabolism

Cytochrome P450s (CYPs) are responsible for about 75% of drug metabolism (12), and their inhibition is a common mechanism of pharmacokinetic DDIs (12). Since each drug in the five DDIs relies on CYPs for elimination, we examined whether the DDIs were possibly caused by inhibition of CYP drug metabolism. Using fluorometric CYP inhibition screening assays, we assessed the potential of the drugs, and their pharmacologically active metabolites, to inhibit the enzymatic activities of the major human CYPs isoforms CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. The half maximal inhibitory concentration (IC_{50}s) are presented in Supplementary Table S6. It is commonly accepted that a dissociation constant (K_i) is more relevant than an IC_{50} when predicting the clinical risk of metabolism-based DDIs. We therefore determined K_is for 11 drug-enzyme pairs (Table 2) that showed relatively strong CYP inhibitions (IC_{50} 20 μM).

Following FDA guidelines for drug interaction studies (13), we applied a stepwise approach to evaluate the risk of clinical DDIs resulting from inhibition of drug metabolism by CYPs. For each of the 11 drug-enzyme pairs for which a K_i was observed, we first used a conservative R-value approach to evaluate each drug's potential to act as a hypothetical

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precipitant. An R-value represents the predicted ratio of the area under concentration-time curve (AUC) of a hypothetical object drug that is exclusively metabolized by the inhibited CYP in the presence vs. absence of an inhibitor. Table 2 shows the predicted R-values. Consistent with FDA guidelines (13), an R value 1.1 (or 11 for CYP3A4 inhibitors administered orally) indicates that the drug could act as a precipitant. With R-values of 1.31 and 1.15, respectively, promethazine and ropinirole could potentially interact with drugs exclusively metabolized by CYP2D6, the isoform most strongly inhibited by both drugs. The predicted potential of the other inhibitor-enzyme pairs was negligible. These determinations suggest that the DDIs not involving promethazine and ropinirole were unlikely to result from inhibition of drug metabolism by CYPs.

A limitation of R values is that they only account for inhibition of a single metabolic pathway without regard to object drugs (14). In cases where multiple pathways are responsible for the metabolism of an object drug, an AUC ratio (AUCR) taking into account the fractional contribution of inhibited pathways to the overall metabolism is preferred. We thus predicted AUCRs for the interaction between ropinirole and loratadine, and that between promethazine and tegaserod. Accounting for 10% of the hepatic metabolism of loratadine by CYP2D6 that would be inhibited by ropinirole (15), the AUCR of loratadine in the presence vs. absence of ropinirole was predicted to be 1.01. Consistent with the FDA guidelines (13), it indicates that loratadine and ropinirole are unlikely to have CYP-based interactions. Because CYP2D6 is insignificant in tegaserod's elimination (16), the inhibition of CYP2D6 by promethazine was considered to have no clinical effect on the pharmacokinetics of tegaserod. Overall, our data suggest that CYP inhibition is unlikely the major mechanism underlying the significant DDIs identified previously.

Inhibition of OATP1B1/1B3-mediated hepatic uptake

It has been increasingly recognized that organic anion-transporting polypeptides (OATPs) represent an important site of DDIs. Particular attention has been paid to OATP1B1 and 1B3, the transporters of OATP family demonstrated as most engaged in drug disposition (17). Among their substrates are many clinically important drugs including simvastatin acid (18), the active metabolite of simvastatin. The risk of simvastatin-induced myopathy was 4.5-fold higher in individuals with a genetic variant of *SLCO1B1* (the OATP1B1 gene), compared to those with the wide type allele (19).

We hypothesized that the DDIs identified previously may result from, at least in part, the inhibition of OATP1B1/1B3 that leads to impaired hepatic uptake and compromised hepatic clearance. We first evaluated the potential of the drugs, as well as their pharmacologically active metabolites, to inhibit the active uptake of β-estradiol 17-β-D-glucuronide ($E_2$17βDG) in cryopreserved rat hepatocytes. $E_2$17βDG is a relatively specific substrate of OATP1B2, a functional homologue of human OATP1B1/1B3 with very similar substrate specificity (17, 20). At 100 μM, simvastatin acid, omeprazole, alprazolam, desloratadine (the active metabolite of loratadine), simvastatin, tegaserod, ropinirole, loratadine and promethazine inhibited $E_2$17βDG uptake by 103.3 ± 0.5%, 60.1 ± 4.8%, 54.5 ± 0.3%, 44.9 ± 14.2%, 36.3 ± 6.0%, 24.6 ± 15.3%, 23.7 ± 2.7%, 18.1 ± 10.9% and 17.7 ± 7.7%, respectively. We then determined the inhibitory potencies of the drugs showing 45% inhibition. The IC_{50}s (95%

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CI) of simvastatin acid, omeprazole, alprazolam and desloratadine were 4.3 μM (3.5, 5.3), 84.3 μM (49.8, 142.9), 99.5 μM (79.5, 124.6) and 140.5 (111.4, 177.1) μM, respectively (inhibition curves are shown in Supplementary Fig. 1).

Following a similar strategy for evaluating CYP-based DDIs, we estimated R-values (from IC_{50}s) to evaluate the drugs' potential to interact clinically with OATP1B1/1B3 substrates. The R-values of simvastatin acid, omeprazole, alprazolam and desloratadine were 3.85, 1.23, 1.01 and 1.01, respectively (Table 3). Consistent with the FDA guidelines(13), simvastatin acid and omeprazole (R value 1.1) might interact with drugs relying on OATP1B1/1B3 for hepatic uptake. The potential of alprazolam and desloratadine as precipitants was negligible.

Direct myotoxicity

Although all the drugs involved in the DDIs have known muscle-related side effects, their direct myotoxicity, except that of simvastatin, has not been examined. We tested whether the DDIs resulted from the direct toxicity of the individual drugs, or their combinations, to muscle cells. We first evaluated the myotoxicity of each individual drug to rat L6 myotubes, a commonly used *in vitro* skeletal muscle model previously used to study mechanisms of statin-induced myopathy (21, 22). After treatment of healthy, fully differentiated myotubes with each drug individually at 10 μM for 5 days, tegaserod, simvastatin, desloratadine and simvastatin acid induced $97.9 \pm 0.4\%$, $73.7 \pm 2.6\%$, $73.3 \pm 1.1\%$, and $33.0 \pm 2.1\%$ myotube death, respectively, compared to DMSO control. The remaining drugs were not myotoxic. We then determined the concentration-effect curves of tegaserod, simvastatin and desloratadine since they induced $> 50\%$ myotube death. The IC_{50}s (95% CI) of tegaserod, simvastatin, and desloratadine were 4.32 μM (4.15, 4.49), 1.64 μM (1.05, 2.56), and 10.94 μM (9.24, 12.96), respectively (Fig. 1a).

The myotoxicity of simvastatin and desloratadine led us to suspect a synergistic interaction that increases risk of myotoxicity when used in combination. We treated myotubes with simvastatin and desloratadine in combination at various concentrations to evaluate their combined toxic effect. The dose response curves of simvastatin shifted leftward with increasing concentration of desloratadine (Fig. 1b). The same trend was observed for desloratadine in the presence of simvastatin (Fig. 1d). Using the method of Chou *et al.* (23), combination index (CI) values were calculated and plotted against fractional myotube death (f_a) (Fig. 1c). Most CI values were less than unity (a few CI values greater than unity near the region $f_a = 0$ likely resulted from methodological flaw (24)), indicating that the interaction between simvastatin and desloratadine was synergistic, such that the drugs notably increased each other's myotoxic effect. This synergistic myotoxicity may contribute to the interaction between simvastatin and loratadine. Direct toxicity to muscle cells, however, was unlikely to explain the other DDIs we identified.

Validation of loratadine-simvastatin interaction

The interaction between loratadine and simvastatin was further validated using an independent dataset, the US FDA Adverse Event Reporting System (FAERS). A distinct feature of the FAERS is that it only includes patients that experience suspected ADRs. As a

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case-only design was considered more appropriate using the FARES, we performed a similar study using the INPC dataset to compare the results. An odds ratio (OR), estimated from a case-only study, is equivalent to a relative risk estimated from a cohort study (25). The ORs are presented in Table 4. Consistent with the RRs presented previously, the concomitant use of loratadine and simvastatin significantly associated with increased risk of myopathy, with ORs of 2.20 (95% CI = (2.02, 3.65)) and 1.53 (95% CI = (1.28, 1.82)) in the FAERS and INPC databases, respectively. In additional subgroup analyses stratified by sex, age, or myopathy type (muscle weakness or myalgia), the interaction between loratadine and simvastatin remained significant in specific subgroups of patients (Supplementary Table S8).

DISCUSSION

Research on pharmacokinetic DDIs traditionally involves prediction of potential DDIs based on molecular mechanistic understanding of the interaction between a drug and its relevant drug-metabolizing enzymes or drug transporters. The clinical importance of hypothesized DDIs is then examined in clinical trials or pharmacoepidemiologic studies. This approach is often limited to a small scope and a relatively low efficiency when used to identify unknown, clinically important DDIs. We sought to overcome these limitations by applying a translational and systematic approach involving pharmacoepidemiologic screening followed by mechanistic investigations.

Our study identified a synergistic myotoxic interaction between simvastatin and loratadine that has never been reported. As simvastatin is one of the most widely prescribed statins, this myopathic interaction could potentially affect a large population. We suggest further studies to confirm this interaction and its myopathic effects. Simvastatin is known to interact clinically with a number of drugs that may further increase its risk of myopathy, including CYP3A inhibitors, such as verapamil, ketoconazole, itraconazole, tacrolimus, erythromycin, clarithromycin, and amiodarone (26, 27) and OATP1B1 inhibitors (e.g., gemfibrozil) (28). Our study, however, did not identify any known DDIs with statins that would increase the risk of myopathy, except for amiodarone (Supplementary Table S5). One possible explanation is that our predefined one-month drug exposure window cannot well capture the concomitance of statins with many CYP3A inhibitors that typically have short exposure. Amiodarone, however, is used chronically and its concomitance with statins is easier to capture. The other explanation is the under-powered interaction analyses between statins and CYP3A inhibitors. Power analysis for these reported DDIs in Supplementary Table S5 showed that almost all of them had less than 10% power, except for the interactions between amiodarone and statins, which had power higher than 70%. Referring back to our initial drug interaction study design, a requested minimum sample size of 100 for two-committed drugs and a minimum of 1.5 risk ratio would give us 65% power in testing the drug interaction effect.

Four out of five DDIs identified in our study involved a commonly used antihistamine, loratadine. Myalgia is one of the side effects of both loratadine and desloratadine (29, 30). Our results suggest that loratadine and desloratadine may be more myotoxic than previously recognized, and can pose even higher risk of myopathy with concomitant use of other drugs.

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The IC_{50}s and K_is that we reported provide a comprehensive view of the potential of these drugs to cause CYP-based DDIs. These data are consistent with those published previously (9). To our knowledge, we are the first to describe the potential of these drugs (except simvastatin) to inhibit OATP1B2 in rat hepatocytes and assess their potential OATP-mediated DDIs in humans. We are also likely the first to report myotoxicity of desloratadine and tegaserod, which may underlie their muscle-related side effects. Of note, simvastatin was much more toxic than simvastatin acid to myotubes *in vitro*, an observation previously reported (31), suggesting that simvastatin-induced myopathy is due primarily to simvastatin rather than simvastatin acid. Similarly, the *in vitro* myotoxicity of desloratadine suggests that myalgia associated with loratadine may be primarily due to its metabolite, desloratadine.

Although inhibition of drug metabolism by CYPs and inhibition of OATP1B1/1b3 are the most common mechanisms underlying pharmacokinetic DDIs, they are unlikely the major mechanisms for the DDIs that we observed. The results from the R-value approach suggest that simvastatin acid and omeprazole may interact with drugs that rely on OATP1B1/1B3 for hepatic uptake. We suggest such data be interpreted with caution, as the R-value approach, for both CYPs and transporters, is known to over-predict the risk of clinical DDIs and lead to spurious conclusions that a drug is a precipitant when it is not (32). It implies, however, that the drug pairs predicted not to interact using this approach in our study are very unlikely to have real interactions.

There are a few limitations to our study. We used a simple cohort design that may be subject to residual confounding and misclassification. The use of the FARES may not provide a definitive validation for simvastatin-loratadine interaction. The CYP450 inhibition assays involve fluorogenic substrates and recombinant CYP enzymes that occasionally generate inhibitory potencies very different from those using conventional approaches. Both the R value and AUCR approaches use a single static *in vivo* concentration of an inhibitor drug, which may overestimate the risk of DDI for drugs, such as simvastatin, with relatively short half-lives and whose circulating concentrations drop rapidly following a dose. We did not evaluate the drugs as direct substrates of OATP1B1/1B3 or other transporters, limiting our understanding of the role of drug transporters in the DDIs. We also used cryopreserved rat hepatocytes and rat L6 myotubes, which are less clinically relevant than human-derived cell models. Future studies are warranted to further evaluate the underlying mechanisms of these DDIs.

METHODS

Evaluation of CYP450 inhibition

Fluorometric cytochrome P450 inhibition kits (BD Biosciences/Gentest, San Jose, CA) were used to determine the IC_{50}s of the drugs for the major CYPs. The assays were performed following the manufacturer's instructions under the conditions in Supplementary Table S7(33). Data were analyzed using GraphPad Prism 5 software (La Jolla, CA).

R values were estimated as $1+ [I]/K_{i,u}$, where [I] is the peak total plasma inhibitor concentration (C_{max}) at the highest proposed clinical dose obtained from the published

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literature, and $K_{i,u}$ is the unbound dissociation constant of the inhibitor. For drugs that inhibited CYP3A4 administered orally, [I] was estimated as $[I] = I_{gut}$ = molar dose/250 mL. AUCRs were predicted using the mechanistic static model in Eq. 1 (34),

$$AUCR = \frac{AUC_{inhibited}}{AUC} = \frac{F_{inhibited}}{F} \frac{1}{\sum_j^n \frac{f_{m,CYP_j}}{1+\frac{[I]}{K_{i,unbound,j}}} + (1 - \sum_j^n f_{m,CYP_j})}, \quad \text{Eq. 2}$$

where $f_{m,CYPj}$ is the fractional metabolism of the object drug through the jth inhibited CYP pathway, $F_{inhibited}$ and F are the bioavailabilities of the object drug in the presence and absence of the inhibitor, respectively. Because $F_{inhibited}$ and F were not available, for conservative prediction, they were assumed to be unity.

Evaluation of inhibition of OATP1B1/1B3

The drugs (100 μM) were incubated with cryopreserved rat hepatocytes (1×10^6 cells/mL) and [^{3}H] $E_2$17βDG (1 μM, 0.1 μCi) for 3 min at 37 °C and 0 °C in triplicate. Uptake was stopped with addition of 1 mL ice-cold PBS and immediate centrifugation at 4500 rpm for 1 min at 4 °C. Cells were resuspended in 1 mL ice-cold PBS and centrifuged again. After removing supernatant, cell pellets were lysed with 200 μL of 50% acetonitrile in H_2O, followed by vigorous vortexing. The fraction of uptake was the ratio of the radioactivity in hepatocyte lysate to the total radioactivity in both lysate and supernatants. The fraction of active uptake was the difference between the total uptake at 37 °C and that at 0 °C.

Evaluation of myotoxicity

Rat L6 muscle cells were cultured as previously detailed by Klip *et al.*(35) with slight modifications. Cells were maintained in monolayer culture in α-MEM containing 10% FBS and 1% antibiotic-antimycotic solution (10,000 U/ml penicillin G, 10 mg/ml streptomycin and 25 mg/ml amphotericin B) in an atmosphere of 5% CO_2 at 37°C. Five days after seeding, myoblasts were differentiated into multinucleated myotubes with 2% FBS. All drug treatments were initiated 5 days after the initiation of differentiation and continued for 5 days. The CellTiter 96® aqueous non-radioactive cell proliferation (MTS/PMS) assay (Promega, Madison, WI) was used to measure cell viability after drug treatment.

Combination index (CI) values were calculated as described by Chou *et al.* (23). The fraction of unaffected (f_u), in this case equivalent to cell viability, was calculated as described above. Fractional inhibition (f_a) was calculated as $1 - f_u$. The slope factor m and IC_{50} of simvastatin and desloratadine were estimated by fitting the data of each drug when applied alone to Eq. 3,

$$\log \frac{f_a}{f_u} = m \times \log(D) - m \times \log(D_m) \quad \text{Eq. 2}$$

CI values were then calculated using Eq. 4,

$$CI=\frac{D_1/(D_1+D_2)}{D_{m_1}(\frac{f_a}{f_u})^{1/m_1}}+\frac{D_2/(D_1+D_2)}{D_{m_2}(\frac{f_a}{f_u})^{1/m_2}} \quad \text{Eq. 3}$$

A CI - f_a plot was constructed by plotting CI values and f_a on y and x axes, respectively.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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STUDY HIGHLIGHT

1. What is the current knowledge on the topic?

Drug-induced myopathy can be exacerbated by DDIs. No study to date has attempted to identify and investigate myopathic DDIs systematically.

2. What question did this study address?

This study identified DDIs that increased risk of myopathy and investigated their underlying mechanisms using a high-throughput, translational approach.

3. What this study adds to our knowledge?

Five previously unknown DDIs were identified to increase the risk of myopathy, none of which appeared to result from inhibition of drug metabolism or hepatic uptake via OATP1B1/1B3. Synergistic myotoxicity may contribute to the interaction between loratadine and simvastatin.

4. How this might change clinical pharmacology and therapeutics?

Pharmacoepidemiologic screening followed by mechanistic investigations proved to be an efficient approach to identify clinically important DDIs.

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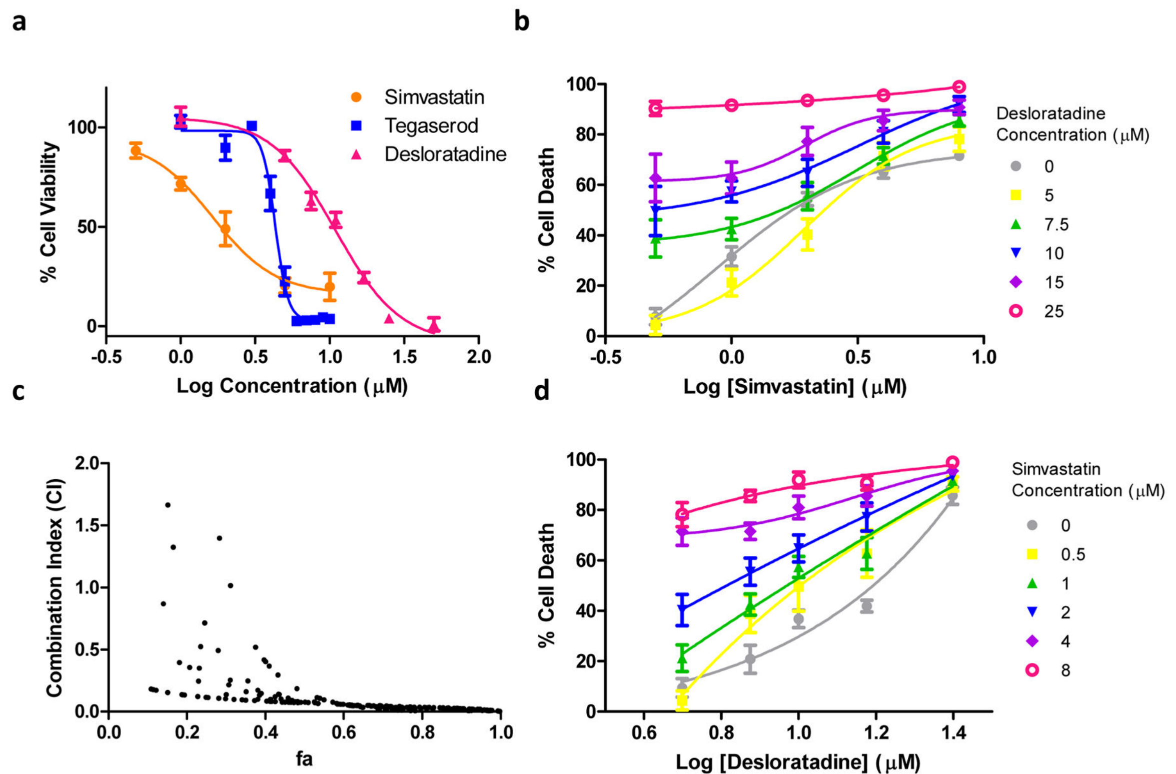

Figure 1.

(**a**) Dose response curves of simvastatin (orange), tegaserod (blue) and desloratadine (pink). Healthy, fully differentiated rat L6 myotubes were treated with individual drugs at various concentrations for 5 days, and myotube viability was determined using MTS/PMS assays. (**b**) Concentration-effect curves of simvastatin in the presence of various fixed concentrations of desloratadine at various concentrations. (**d**) Concentration-effect curves of desloratadine in the presence of various fixed concentrations of simvastatin. (**c**) Combination index (CI) – fractional myotube death (f_a) plot. CI = 1 indicates additivity (no interaction). The points above 1 indicate antagonism and those below indicate synergism.

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Table 1

Drug-drug interactions associated with increased risk of myopathy after adjusting for age and sex

Drug1	Drug2	Risk1	Risk2	Risk12	Risk Ratio (95% CI)	M1	N1	M2	N2	M12	N12
Loratadine	Simvastatin	0.022	0.033	0.093	1.69 (1.39, 2.06)	1,264	44,245	4,197	102,345	137	1,223
Loratadine	Alprazolam	0.022	0.029	0.095	1.86 (1.50, 2.31)	1,257	43,341	2,251	52,341	176	1,448
Loratadine	Ropinirole	0.020	0.018	0.122	3.21 (2.06, 5.00)	1,218	43,491	164	6,531	17	123
Promethazine	Tegaserod	0.011	0.020	0.093	3.00 (1.94, 4.64)	1,332	78,334	109	3,745	23	224
Loratadine	Omeprazole	0.022	0.059	0.102	1.26 (1.15, 1.38)	1,260	44,207	4,339	70,345	304	2,734

Risk1 and risk2 are myopathy risks for drug 1 and drug 2, respectively. The risk ratios were calculated as risk12/(risk1+risk2). 95% CIs were calculated using multivariate logistic regression adjusted for age and sex. N1, N2 and N12 is the number of patients who had prescription for drug 1 only, drug 2 only, and both drugs, respectively; and M1, M2 and M3 is the number of myopathy cases who had prescription for drug 1 only, drug 2 only, and both drugs, respectively.

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Table 2

Predicting potential of CYP-based drug-drug interaction.

Inhibitor	Pathway	Dissociation Constant (K_i, μM)	Fraction of Unbound ($f_{u,inc}$)	Unbound Dissociation Constant ($K_{i,u}$, μM)	Peak Plasma Concentration (C_{max}, ng/ml)	Inhibitor Concentration ([I], μM)	Predicted R-Values
Simvastatin	CYP3A4	0.51	0.93	0.47	-	0.764	2.61
Promethazine	CYP2D6	0.25	0.88	0.22	19.3 (36)	0.068	1.31*
Tegaserod	CYP3A4	5	0.92	4.61	-	0.796	1.17
Ropinirole	CYP2D6	0.85	0.84	0.71	26.9 (37)	0.103	1.15*
Loratadine	CYP2D6	0.5	0.93	0.47	4.12 (38)	0.011	1.02
Tegaserod	CYP2D6	0.51	0.92	0.47	2.7 (39)	0.009	1.02
Loratadine	CYP2B6	2	0.93	1.86	4.12 (38)	0.011	1.01
Simvastatin	CYP2C9	18.3	0.93	17.03	25.4 (40)	0.061	1.00
Loratadine	CYP2C9	7.6	0.93	7.07	4.12 (38)	0.011	1.00
Tegaserod	CYP2C19	9.2	0.92	8.48	2.7 (39)	0.009	1.00
Tegaserod	CYP2C9	11.4	0.92	10.51	2.7 (39)	0.009	1.00

K_i is the dissociation constant determined *in vitro*; $f_{u,inc}$ is the fraction of unbound drug in the incubation mixture and was predicted using the Hallifax-Houston model (41); $K_{i,u}$ is the unbound dissociation constant estimated as $K_i * f_{u,inc}$; C_{max} is the peak total plasma concentration at the highest clinical dose; [I] is the inhibitor concentration used to predict R values and is equal to C_{max}, except for CYP3A4 inhibitors administered orally. For simvastatin and tegaserod with CYP3A4, [I] is the estimated gut concentration at the highest proposed clinical dose, 80 mg (191 μM) and 6 mg (19.9 μM), respectively, divided by 250 mL (approximate gut volume); R values were estimated as $1 + [I]/K_{i,inc}$;

*denotes R values 1.1 (or 11 for simvastatin and tegaserod with CYP3A4), indicating a probable clinical CYP450-based DDI.

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Table 3

Predicting potential of OATP1B1-based drug-drug interaction.

Drug	Half Maximum Inhibition Concentration (IC_{50}, μM)	Dose (mg/mmol)	Molecular Weight (g/mol)	Peak Plasma Concentration (C_{max}, μM)	Maximal Hepatic Inlet Concentration ($[I]_{inlet,max}$, μM)	Predicted R Value
Simvastatin Acid	4.3	80/0.183	436.6	0.058 (40)	12.274	3.85
Omeprazole	84.3	80/0.232	345.42	4.146 (42)	19.586	1.23
Alprazolam	99.5	3/0.01	308.76	0.333 (43)	0.981	1.01
Desloratadine	140.5	5/0.16	310.8	0.015 (44)	1.088	1.01

Dose is the highest proposed clinical dose; C_{max} is the peak plasma concentration at the highest proposed clinical dose; $[I]_{inlet,max}$ was estimated as C_{max} + (k_a x Dose x F_aF_g/Q_h) (13), where Q_h is the hepatic blood flow (1500 mL/min), k_a is the absorption rate constant, and F_aF_g is the fraction of oral dose that reaches the liver. Because the values of k_a and F_aF_g were not available, for conservative predictions, they were assumed equal to the theoretical maxima of 0.1 min^{-1} and 1 (13), respectively. R values were estimated as 1 + $[I]_{inlet,max}/K_i$. Because the concentration of $E_217\beta DG$ (1 μM) was well below its K_m (45, 46), the K_is were approximated by the IC_{50}s based on $K_i = IC_{50}/(1 + [S]/K_m)$ (47). For simvastatin acid, the C_{max} and dose were assumed equal to those of simvastatin. For desloratadine, R values were estimated with the C_{max} following the highest clinical dose of desloratadine since it is higher than the C_{max} following that of loratadine (48, 49).

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Table 4

Testing and Validation of the loratadine/simvastatin interaction using case-only datasets

Data Sets	Odds Ratio	95% CI	N12	N1	N2	N00
INPC CDM	1.53	(1.28, 1.82)	37	1,264	4,197	5,572
FAERS	2.20	(2.02, 3.65)	37	276	6,116	100,531

N12, N1, N2 and N00 is the number of myopathy cases with prescription for both simvastatin and loratadine, simvastatin only, loratadine only, and neither drug, respectively. INPC CDM stands for Indiana Network of Patient Care Common Data Model. FAERS stands for the FDA adverse event reporting system