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Systems Pharmacology of Adverse Event Mitigation by Drug Combinations

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Data and materials availability: Code files are provided as a supplementary document (CodeFiles.pdf). The data for this study can be found in the FAERS, and MSDW data can be obtained freely with proper institutional review board approval by contacting O.G. Confounding variable analysis relating to Table 2 and additional information on drug combinations that mitigate adverse events can be found at the SBCNY Web site (<http://www.sbcny.org>).

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/5/206/206ra140/DC1

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File "table S14.xlsx" (Exhaustive list of drug B mitigation of drug A-associated adverse events in FAERS)

File "CodeFiles.pdf" (computer code for analysis of FAERS)

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Abstract

Drugs are designed for therapy, but medication-related adverse events are common, and risk/benefit analysis is critical for determining clinical use. Rosiglitazone, an efficacious antidiabetic drug, is associated with increased myocardial infarctions (MIs), thus limiting its usage. Because diabetic patients are often prescribed multiple drugs, we searched for usage of a second drug (“drug B”) in the Food and Drug Administration’s Adverse Event Reporting System (FAERS) that could mitigate the risk of rosiglitazone (“drug A”)–associated MI. In FAERS, rosiglitazone usage is associated with increased occurrence of MI, but its combination with exenatide significantly reduces rosiglitazone-associated MI. Clinical data from the Mount Sinai Data Warehouse support the observations from FAERS. Analysis for confounding factors using logistic regression showed that they were not responsible for the observed effect. Using cell biological networks, we predicted that the mitigating effect of exenatide on rosiglitazone-associated MI could occur through clotting regulation. Data we obtained from the *db/db* mouse model agreed with the network prediction. To determine whether polypharmacology could generally be a basis for adverse event mitigation, we analyzed the FAERS database for other drug combinations wherein drug B reduced serious adverse events reported with drug A usage such as anaphylactic shock and suicidality. This analysis revealed 19,133 combinations that could be further studied. We conclude that this type of crowdsourced approach of using databases like FAERS can help to identify drugs that could potentially be repurposed for mitigation of serious adverse events.

INTRODUCTION

Drugs have both therapeutic and adverse effects (1). A general goal in pharmacology is to optimize the therapeutic efficacy while reducing the adverse event risks. Traditionally, this is done through medicinal chemistry by altering drug structure (2). Attempts have also been made to reduce adverse events by tailoring the choice of drug or dose to an individual patient’s genomic status (3, 4). Neither approach works consistently owing to the complex physiological relationships underlying drug action. Because drug targets are nodes within cellular regulatory networks (5, 6), there may be intrinsic coupling between therapeutic and adverse effects. To separate the two effects, we need to focus on the target and its interactions within the networks underlying the physiological functions associated with the therapeutic and adverse effects. A second drug at another target may mitigate the adverse events of the first drug through network interactions.

Often drug combinations are used to minimize adverse effects—for example, the use of atropinics to minimize the muscarinic adverse effects of cholinesterase inhibitors that are used for expedited recovery from nondepolarizing neuromuscular blockers (7). In a case like this, the targets for the protective drugs are predictable on the basis of the mechanisms of adverse effects of the primary agent. We hypothesize that there may be many such drug pairs where one drug reduces the adverse effects of the other while maintaining efficacy. If we can identify such drug pairs, an analysis of the networks to which the drug targets belong may help us develop strategies to decouple therapeutic and adverse effects. To find such targets, we first identified drug combinations that result in decreased adverse event incidences. Databases, such as the Food and Drug Administration’s (FDA) Adverse Event

Reporting System (FAERS), that link drug usage to adverse events provide a rich, albeit imperfect, and empirical source to find for such drug combinations.

The FAERS database contains millions of records of drug-induced adverse events for both single and combination therapies generated by individual reports from patients, physicians, hospitals, lawyers, and drug companies. FAERS has allowed us to identify unknown drugs and targets associated with long QT syndrome (8). Others have used this database to identify drug combinations that lead to unanticipated adverse events and developed methodologies to effectively mine this database (9). Although there are limitations of the FAERS that preclude definitive conclusions, it is a potentially useful, freely available, large data set maintained by the U.S. government. Hence, we decided to analyze FAERS, not as an end in itself, but to generate polypharmacology hypotheses that can be tested in animal models or prospective clinical trials. Theoretically, we should be able to identify not only adverse but also beneficial drug combinations from FAERS. This allows us to ask the question: Can we use FDA-approved drugs for adverse events reduction? To answer this question, we looked for combinations where “drug B,” when taken with “drug A,” reduces reports of serious adverse events from patients taking drug A. In short, FAERS analysis can be used as a hypothesis generator for drug combinations that could be tested in animal models or clinical trials.

We focused on rosiglitazone, a drug that had been widely used for effective control of blood glucose in type 2 diabetes patients (10). Despite its therapeutic efficacy, rosiglitazone use has declined in both the United States and Europe because of increased risk of myocardial infarctions (MIs) and stroke (11, 12). Our search of the FAERS showed that exenatide (drug B), when administered with rosiglitazone (drug A), reduced the number of MI and stroke adverse effects reports associated with rosiglitazone usage. We developed cell biological networks from molecular interaction databases to identify and rank potential pathophysiological mechanisms and tested the preferentially ranked mechanism in an animal model. Further analysis of the FAERS indicated that the beneficial effects of drug combinations are not limited to exenatide and rosiglitazone. Nineteen thousand one hundred thirty-three combinations were identified where drug B appears to reduce adverse events associated with drug A for a varied range of adverse events. These observations point to FAERS as a potential low-cost source of human data for identification of beneficial combinations, and thus increase the therapeutic index of currently approved drugs.

RESULTS

Statistics of MI reports in clinical databases

The approach in this study is shown in Fig. 1A. We analyzed the FAERS to estimate the association of rosiglitazone usage with a serious adverse event, MI, and how the co-usage of a second drug affected the percentage of MI reports. Exenatide usage was associated with the greatest reduction in percentage of MI reports, from ~34% MI reported without the drug to ~2% when using exenatide (odds ratio, 0.04) (Fig. 1B). Because FAERS contains only adverse events and does not provide estimates of the total number of individuals who used the drug, we used the odds ratio determination under varying reporting categories as an approach to estimate the significance of MI association with rosiglitazone. The flow chart for this analysis is shown in Fig. 2A.

Patient reports ($n = 3,918,732$) were split into multiple categories: type 2 diabetic/non-type 2 diabetic, metformin/all other drugs except metformin, or rosiglitazone/all other drugs except rosiglitazone; we then evaluated the significance of reported MI odds ratios when exenatide was coprescribed. We estimated the fraction of MI as adverse events for the total number of reported adverse events associated with all drug usage. For reports without

rosiglitazone, MI was 2.1% of all adverse event reports, and for reports with rosiglitazone, MI was 31.7% (Fig. 2B). In contrast, MI was 4.3% of reports with metformin compared to 2.5% of adverse event reported for all drugs other than metformin (Fig. 2B). MI was 2.9% of reports for patients with type 2 diabetes (Fig. 2B). All of these differences were significant. In FAERS, exenatide usage resulted in a preferential decrease in the percentage of reported MI for patients prescribed rosiglitazone, and MI accounted for only 2.13% of all reported adverse events associated with exenatide + rosiglitazone (odds ratio, 0.04) (Fig. 2C). A decrease in the MI reporting percentage for metformin + exenatide and type 2 diabetes + exenatide was also observed at 0.94% (odds ratio, 0.17) and 0.68% (odds ratio, 0.14), respectively (Fig. 2C). The odds ratio for MI reporting for all reports in FAERS with exenatide (406 of 49,129) versus all reports in FAERS without exenatide (99,780 of 3,869,603) was 0.31 [95% confidence interval (CI), 0.28 to 0.34; $P < 0.01$, evaluating the odds ratio based on the log-normal distribution, as described in Supplementary Methods].

We also analyzed anonymized patient records in Mount Sinai Data Warehouse (MSDW). In this database, we were able to retrieve the total number of patients prescribed rosiglitazone and the number of MI incidents in this cohort. Of patients prescribed rosiglitazone, 8.4% suffered MI compared to 2.1% for all patients in the MSDW (Fig. 2B). There were only 34 patients in MSDW who received rosiglitazone and exenatide, and only 1 suffered MI (Fig. 2C). The extremely limited sample size of these observations precludes any robust statistical analysis or conclusions. Despite these known limitations, we nonetheless cautiously note a similarity to that seen in the FAERS. A detailed list of other drugs used in combination with rosiglitazone that had significantly decreased the incidence of MI reports in FAERS is given in table S1.

Analysis for confounding variables

The effect shown in Fig. 2 could arise for several reasons. We conducted extensive cofactor analysis to rule out various confounding factors. Covariates such as gender (table S2) and occupation (table S3) of the reporting individual did not affect the significance of exenatide effect. We compared the effect of exenatide on MI for patients receiving only rosiglitazone and not metformin as well as patients receiving only metformin and not rosiglitazone (table S4), where we found that the effect was preferential for rosiglitazone. Using MeDRA (Medical Dictionary for Regulatory Activities) terms (table S5), we directly compared patients receiving rosiglitazone treatment to patients who did not receive rosiglitazone treatment, and found a significantly different odds ratio and percent reduction of MI when exenatide was coprescribed (table S6).

Usage of a third drug (tables S7 and S8), the occurrence of other adverse events (tables S9 and S10), age of the patient found in the report (fig. S1), and time of filing the FAERS report (fig. S1) were not factors that affected the observed effect. When we examined the covariates in a multivariate logistic regression model (table S11), we observed a significant contribution by exenatide ($P < 10^{-16}$, mean and SE determined through logistic regression and evaluated under a normal distribution). When this model was compared to a multivariate logistic regression model excluding exenatide as a factor (table S11), using a likelihood ratio test, we observed a significant improvement when exenatide was included. The null model has a log likelihood score of -33,738 (table S12), and the model including exenatide has a log likelihood score of -33,645 (table S11). The D value is 186 for one degree of freedom at $P < 10^{-16}$.

A potentially important factor that may influence the large percentage of adverse event reports of MI for rosiglitazone is the bias introduced by reporting entities after the study by Nissen and Wolski (11) was published in 2007. When we focused on FAERS reports before June 2007, we obtained an odds ratio of 1.28 for reports where rofecoxib was excluded

(table S13). The odds ratio of 1.43 (95% CI, 1.03 to 1.98; $P = 0.03$, evaluating the odds ratio based on the log-normal distribution, as described in Supplementary Methods) in (11), using metadata from clinical trials, overlaps with our odds ratio estimated from FAERS. It is also noteworthy that despite the sharp increase in reporting of rosiglitazone-associated MI after June 2007, the reduction of odds ratio for MI reports by exenatide remains similar: odds ratio of 0.07 before (11), and odds ratio of 0.07 after (11) (table S13).

Subnetworks associated with rosiglitazone-induced MI

We sought to identify potential molecular mechanisms by which rosiglitazone could increase the incidence of MI. For this, we developed cell biological interaction networks that included the rosiglitazone target peroxisome proliferator-activated receptor γ (PPAR γ), which is a transcription factor. These subnetworks were developed to connect PPAR γ to potential pathophysiology underlying MI. We considered two pathophysiological processes that can lead to MI (and also stroke): regulation of fluid retention by the kidney and blood clot formation. PPAR γ regulates the carbonic anhydrase gene (13). This could lead to increased fluid retention and potentially to hypertension leading to MI (12). This is an attractive hypothesis because it is parsimonious in terms of network interactions, and it provides a simple explanation of how the same drug target in different organs can have therapeutic and adverse effects at the organismal level. We computationally tested if regulation of fluid retention was a plausible hypothesis using Fisher's exact test. Our approach is schematically described in fig. S2. For this, we developed "fluid retention" and "clotting regulation" subnetworks, starting with PPAR γ as the seed node. The respective subnetwork contained proteins that can modulate fluid retention or clotting and were separated by one intermediate node from PPAR γ .

We analyzed FAERS to identify drug B possibilities that modulate MI for rosiglitazone-treated individuals. Seventy-five drugs were identified using a log odds ratio cutoff Z score of 2, using 5000 individuals. We then determined how many of these drug B targets were present in each network. In evaluating these networks, we assumed that the targets for drug B are based on the targets listed in DrugBank 2.0. However, we did not assume that the targets for drug A and drug B need to interact directly. The effects could be due to the downstream convergence of the pathways from drug A and drug B. We determined that targets of 75 drugs in FAERS that alter rosiglitazone-associated MI reports did not significantly overlap with the list of nodes within our fluid retention network ($P = 0.062$, Fisher's exact test) (fig. S3). In contrast, when we conducted a similar analysis using the clotting regulation network, we identified a significant overlap ($P = 0.016$, Fisher's exact test). Together, the data in Figs. 2 and 3 suggest that regulation of clotting might be a relevant pathophysiology underlying the interaction between rosiglitazone and exenatide. The clotting subnetwork predicts plasminogen activator inhibitor-1 (PAI-1) as a point of convergence between rosiglitazone and exenatide.

Convergence of rosiglitazone and exenatide effects on PAI-1 in *db/db* mice

PPAR γ can induce an increase in PAI-1 levels in human endothelial cells (14), and exenatide inhibits tumor necrosis factor- α -induced production of PAI-1 in human endothelial cells (15). To investigate whether exenatide may have similar effects on rosiglitazone-induced PAI-1 levels, we evaluated drug interaction in vivo using spontaneously diabetic (*db/db*) mice and genetically matched wild-type mice as controls. This model was chosen because the diabetic mice provide a similar systemic background to test the effect of rosiglitazone and exenatide. Further, because risk of MI and stroke are often associated with clotting propensity in humans, this animal model system allows us to test if the rosiglitazone-exenatide interaction converges at the regulation of clotting.

No effects of rosiglitazone alone or rosiglitazone + exenatide on PAI-1 levels in wild-type mice were observed (Fig. 4A). The diabetic mice and wild-type mice had no significant differences in PAI-1 levels ($P = 0.11$). Treatment with rosiglitazone resulted in a 74% increase in PAI-1 levels at the end of the 10-week period compared to untreated diabetic animals ($P < 0.01$). Exenatide-treated *db/db* mice did not have significantly lower PAI-1 levels ($P = 0.26$) when compared to untreated *db/db* mice (Fig. 4B). However, in the presence of rosiglitazone, exenatide significantly decreased PAI-1 levels ($P < 0.01$) such that it was not statistically different from untreated *db/db* mice ($P = 0.68$). No significant body weight differences were observed between animals treated with rosiglitazone + exenatide and rosiglitazone alone (Fig. 4B) at the end of the study ($P = 0.35$). Rosiglitazone significantly reduced plasma glucose ($P = 0.02$), and this was not further affected by exenatide (Fig. 4B) ($P = 0.20$). For all tests of significance in Fig. 4, we used a two-tailed, unpaired t test.

Effect of exenatide on thrombogenesis and cardiac function in rosiglitazone-treated *db/db* mice

We studied the effects of rosiglitazone and rosiglitazone + exenatide on the clotting characteristics in diabetic animals. Because clinical studies indicate a complex relationship between PAI-1 levels and pathophysiology in patients treated with rosiglitazone (16), we analyzed various facets of clotting. We performed thromboelastometry analysis on whole blood from mice treated with rosiglitazone alone as well as mice treated with both rosiglitazone and exenatide to determine clotting time (CT; the time from initiation of the test to formation of a 2-mm clot), clot formation time (CFT; the time elapsed between 2- and 20-mm clot firmness), and maximum clot firmness (MCF; the peak size of the clot in millimeters). This assay measures multiple characteristics and hence was preferable to thrombogenesis assay when we needed to understand the nature of the modulatory interactions regulating clot characteristics, such as effects of changing PAI-1 levels. However, because this assay is quite variable from run to run, comparisons were always examined in a pairwise fashion on the basis of a run of each sample set from the four different conditions at the same time.

CT was not significantly different between rosiglitazone- and rosiglitazone + exenatide-treated animals (Fig. 5). This observation is in accordance with the lack of effect of clopidogrel in reducing rosiglitazone-associated MI reports in humans (odds ratio, 0.92; 95% CI, 0.83 to 1.02; $P = 0.12$) because this drug modulates platelet aggregation to affect initial CT (17). In contrast, CFT and MCF were significantly affected (Fig. 5A). Figure 5B shows the comparison of clotting characteristics of blood from control (saline-injected) and rosiglitazone-, exenatide-, and rosiglitazone + exenatide-treated *db/db* mice. CFT and MCF are markers indicative of kinetics and firmness of the clots. The effects suggest a significant reduction in clot dynamics in animals receiving rosiglitazone + exenatide when compared to rosiglitazone monotherapy. For all tests of significance in Fig. 5, we used a one-tailed, paired t test.

We measured fractional shortening by echocardiography. Overall, no significant differences were found (Fig. 5C). However, several animals receiving rosiglitazone monotherapy showed levels of reduced fractional shortening not seen in the other groups. Together, the reported human data and our in vivo data in the diabetic mouse indicate that the administration of exenatide for type 2 diabetes patients treated with rosiglitazone reduces the percentage of MI reports, and this reduction seems to be mediated by modulating clot dynamics.

Detection of additional beneficial drug combinations in FAERS

Rosiglitazone and exenatide are used to treat the same pathophysiology. We determined whether the drug combinations in FAERS would provide beneficial effects even when they were used to treat different pathophysiologies. We identified 19,133 combinations where poly-pharmacology could potentially reduce the frequency of reporting of adverse events by drug A when drug B was coprescribed. A select list in Table 1 shows that the adverse events are varied and include serious ones such as anaphylactic shock and suicide. Sometimes, the mitigating effect was a drug class–drug class interaction; in other cases, only some drugs within a class displayed the behavior of interest. We provide an exhaustive list of interactions in which drug B mitigated the adverse event potential associated with drug A in table S14. Sometimes, both drugs were prescribed for similar pathophysiology and in other cases for different pathophysiologies. Significances were determined on the basis of odds ratio as described in Materials and Methods.

DISCUSSION

An individual can be affected by multiple pathologic conditions that require the administration of several drugs or requires more than one drug for the same condition. In many instances, the drugs have no unanticipated interactions producing the expected therapeutic effects. However, there are many cases where drug combinations produce unanticipated adverse events. We can envisage three types of poly-pharmacology effects: (i) each drug is efficacious in treating the intended disease, and they do not interact; (ii) the drug combination results in unanticipated interactions leading to undesirable side effects (18); (iii) there is an unanticipated beneficial effect of drug combination whereby a second drug (drug B) leads to a mitigation of adverse effects produced by the first drug (drug A). Just as it is possible to discover harmful side effects through retrospective examination of FAERS (9), we show here that we can detect potential beneficial combinations by analysis of FAERS.

Combining statistical models of drug interactions from FAERS with cell biological interaction–based subnetwork construction can lead to generation of unbiased hypotheses for mechanisms of action. We focused on rosiglitazone, which is associated with MI and stroke (11). Using FAERS, we confirmed that rosiglitazone usage led to increased MI reports. We then searched within the FAERS in an unbiased manner for a drug B to best reduce these adverse event reports. The use of a second drug allowed us to look for the difference (adverse event) within a difference (drug B) for the overall group (patients prescribed drug A). This type of an approach has been successfully used in economics (19), and its limitations have been examined (20). Through this analysis, we found that exenatide significantly reduces reporting of both MI and stroke. Because exenatide works well clinically to reduce blood glucose with metformin or rosiglitazone, coprescription of exenatide with rosiglitazone could be more beneficial than coprescription of warfarin or other anticoagulants that have no effect on blood glucose, although this has to be balanced with increased risk of pancreatitis associated with exenatide (21).

To identify mechanisms of drug interaction, we used the human interactome to construct cell biological networks based on drug targets as seed nodes. These subnetworks can be used to develop hypotheses for experiments in animal models. We used statistics of network analysis to identify clotting regulation as a preferred potential mechanism and then tested this in an animal model of diabetes. Combining network analysis with previous knowledge, we identified PAI-1 as a potential locus of combined regulation by exenatide (15) and rosiglitazone (16). Experiments in the *db/db* mouse model indicate that exenatide suppresses rosiglitazone-induced increases in levels of PAI-1 and clotting. Rosiglitazone has been reported to decrease PAI-1 levels in humans after insulin injection, because insulin increases

PAI-1 level (22). In our study, the effect of rosiglitazone increases PAI-1 in diabetic animals that have not been given exogenous insulin. This seemingly contradictory effect of rosiglitazone in regulating PAI-1 suggests that it may act to stabilize PAI-1 levels around at a certain level (such as 30 ng/ml in humans and mice) rather than decreasing or increasing PAI-1 levels universally (16). However, this phenomenon was not further examined in this study.

When monotherapy results in incomplete therapeutic response, as is the case for diabetics treated with rosiglitazone, a second drug such as exenatide is used to control levels of blood glucose. In addition to this intended therapeutic effect, our study shows that there is an additional unintended benefit: mitigation of a serious adverse event. As shown in Table 1 and table S14, multiple classes of drugs may have the ability to mitigate adverse events induced by other drugs. However, each of these combinations identified through analyses of FAERS has to be tested in animal model experiments and/or clinical trials to establish the beneficial effects identified prospectively.

The data in large databases are continuously improving. However, large databases like FAERS will have limitations. As a result of their unsupervised gathering, it is likely that some of the data are inaccurate. Further, the lack of appropriately matched controls will always limit the certainty with which the conclusions can be considered. Nevertheless, these are valuable primary human data, obtained under real-life situations, and hence, there should be an appropriate perspective on their value. Consequently, ruling out confounding variables will always be essential. A standardized universal electronic medical record system that contains data from many academic medical centers with systematically recorded clinical phenotypes, real-time updates, and associated molecular and genomic data would be useful for studies such as this one. When used in conjunction with FAERS in understanding drug-drug interactions, it could resolve many confounding variables.

Animal models also can be useful in providing mechanistic insight into complex pathophysiologies and drug interactions and thus ruling out confounding variables. However, such animal model studies for drug interactions have their own limitations. Sometimes, the regulatory topology and physiological functions in animals are different from humans (23). These differences can be manifested in variations in drug responses, thus confounding usage in humans.

Newer iterations of our method using well-constructed clinical databases that are integrated with genomic characteristics could improve sensitivity and specificity for identification of drug combination usage for both harmful and beneficial effects in defined populations under particular societal and environmental conditions. These computational predictions could then be tested in human pharmacoepidemiological trials. It is hoped that this type of analysis will lead to many currently used drugs being repurposed for mitigation of serious adverse events in individual patients. In order for these potential beneficial drug combinations to be routinely used, two types of follow-ups are needed. First, in animal models, the basic mechanisms by which the drug combinations have their effect have to be established. Second, prospective clinical trials need to be conducted to establish that the proposed combinations have the intended effects under controlled conditions.

MATERIALS AND METHODS

Study design

The overall goal of this study was to identify beneficial drug combinations from the publicly available FAERS database. We hypothesized that drug combinations, in addition to evoking new adverse events not associated with either drug, could also evoke beneficial effects

suppressing adverse events evoked by an individual drug. We included all available data within FAERS on drugs and adverse events. Because only adverse events are reported in FAERS, the total number of users for any drug is not directly available. To compensate for this, we used the total number of adverse event reports associated with a drug of interest as a denominator. Use of this approach precluded the need for randomizing adverse events or drug usage. Because the computational studies were retrospective, no blinding was needed. For animal experiments, the biochemical and physiological measurements were made in a blinded fashion.

Analysis of FAERS for drug combinations

FAERS contains 3,918,732 unique incident reports from January 2004 to March 2012. We converted the names of the drugs to their generic names through a previously published dictionary (8). We then examined the top-ranked drugs that were associated with decreasing the MI reports for patients prescribed rosiglitazone. As background cases, we used all incidents when any first drug was used, and the second drug of interest was not used (for example, exenatide). We selected for drugs that significantly reduced the odds ratio of MI adverse event by 2 SEs by scaling for 5000 patient data sets, which was empirically selected as a “pharmacoepidemiology” study. A detailed description of both the odds ratio calculation and trial size scaling can be found in the Supplementary Materials.

Analysis of Mount Sinai Medical Center electronic medical record data

We supplemented the FAERS analysis using longitudinal data in the MSDW, which contains longitudinal clinical and administrative data for about 3 million patients who have undergone care at the Mount Sinai Medical Center, New York. Within this database, we selected for patients who had at least one encounter per year between 2009 and 2011 to ensure that we had both recent and sufficient data on all individuals included in the analysis. This selection yielded a set of 173,655 patients for further analysis. Using the entirety of the longitudinal medical record for these individuals, we selected for patients who were treated with any form of rosiglitazone, noting the first recorded date of prescription for each patient. To assign the presence or absence of certain outcomes in individuals treated with rosiglitazone, we used the ICD-9 (International Classification of Diseases, 9th Revision) codes 410* to denote “acute myocardial infarction.” Others have validated the use of these ICD-9 codes for analyses using both clinical and administrative databases and have found the positive predictive value to generally exceed 0.95 (24, 25). The sensitivity of outcome codes can be highly variable and difficult to assess; however, in our experience, the MSDW being a clinical database and the application of a criterion requiring at least 3 years of recent contiguous visits increases the sensitivity of outcome ICD-9 codes in a selected cohort. For this analysis, the first incidence of the ICD-9 code was taken to be the date for the event of interest, and if the first date of rosiglitazone prescription preceded the event date, then the patient was deemed to have had an event while taking rosiglitazone. We evaluated the odds ratio for MI for individuals treated with and without rosiglitazone. We also evaluated the odds ratios for MI and exenatide based on patients where rosiglitazone was given. The odds ratio for MSDW was evaluated in the same way as for FAERS.

Network construction and identification of drug effect

We used an updated version of a previously described human interactome (8). The method for generating cell biological networks is based on protein-protein interaction databases as referenced in (8) along with a gene regulatory database (26), and it is described in detail in Supplementary Methods. Because genes and their products (that is, proteins) are not separately treated as nodes, these networks are composite protein-protein interaction and gene regulatory networks, which we call cell biological networks. From these interactions, we curated two subnetworks. Using the neighboring nodes within the Gene Ontology

categories (27) of “multicellular organismal water homeostasis” or “excretion,” we created the “fluid regulation” subnetwork. Using the term “regulation of blood coagulation,” we created the “clotting regulation” subnetwork. These are subnetworks of the human interactome, which implies that they contain only a subset of nodes and edges from the entire human interactome. A more detailed explanation of this process is given in Supplementary Methods.

Analysis of FAERS for adverse event–mitigating drug combinations

For an exhaustive analysis of drug-drug combinations, we identified any drug B that had a significant reduction of reporting of the top 25 ranking adverse events associated with drug A. For this analysis, we defined the following criteria: (i) minimum of 500 adverse events for any drug combination; (ii) drug A should have more than 200 reports of the adverse events; and (iii) there is a reporting odds ratio difference of at least 2 SEs for a patients data set of 5000 with and without drug B. The values in these criteria were empirically chosen. Relaxing these values can be used to obtain more combinations; however, this will not affect the statistics of the combinations shown here. Following this analysis, we identified particular drugs or drug classes (composed of multiple individual drugs) of interest and analyzed the reporting odds ratio for specific adverse events associated with drug set A with and without drug set B.

Determining the interaction subnetwork between two drugs

To optimize the interaction subnetwork between the two drugs, we used a random walk–based algorithm. This approach identifies nodes that would be preferentially affected by specific interactome perturbations by drugs binding to their known target in DrugBank 2.0 when compared to perturbations of the same magnitude at any node in the human interactome. To accomplish this, we estimated the biased probability of reaching a particular node of interest because random walks are taken from the specified drug targets compared to starting from any node within the interactome. A detailed description of this can be found in Supplementary Methods.

Treatment of animals

All animal protocols were approved by the Institutional Animal Care and Use Committee at Icahn School of Medicine at Mount Sinai. *db/db* [B6.BKS(D)-Lepr^{dp/J}], and wild-type (C57BL/6J) mice were purchased from The Jackson Laboratory. They were kept with dark-light cycle from 7 a.m. to 7 p.m. at the school’s animal facilities with free access to food and water. Animals were randomly assigned to individual groups. Rosiglitazone (10 mg/kg per day) or control (saline supplemented with 5% dimethyl sulfoxide and 0.3% bovine serum albumin) were sterile-filtered and administered intraperitoneally. Exenatide (ex4) was purchased as a powder from Tocris or in long-lasting form (ex4) (bydureon) from Amylin Pharmaceuticals LLC. A suspension of ex4 was prepared according to the manufacturers’ instructions. For the regular ex4, the dosage was 20 µg/kg per day, and for long-lasting ex4, the dosage was 27 µg/kg per week, both administered intraperitoneally. Rosiglitazone dosages were determined empirically on the basis of adequate blood glucose control in *db/db* mice, and ex4 dosage was calculated on the basis of the prescribed human dosage, assuming the average human has a body weight of 75 kg. Blood (50 µl) was collected once a week from the tail vein with a heparin-coated capillary. The blood samples were immediately mixed with 5 µl of 0.1 M sodium citrate and centrifuged at 3000g for 10 min. Plasma samples were numerically labeled and stored at –20°C until use.

Statistical analyses

Drug combination identification—We identified drug combinations in FAERS and MSDW for adverse event occurrence or mitigation by using the odds ratios. This was evaluated by examining their 95% CIs assuming log-normal distributions. The SE of the distribution is given by the square root of the sum of the occurrences provided within the confusion matrix as defined by the intersection of the two drug treatment groups and two adverse event groups. The CI was evaluated with the following formula:

$$\ln \left(\frac{a/c}{b/d} \right) \approx N \left(0, \frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d} \right)$$

We performed logistic regression using the Newton-Raphson method, solving for the coefficients for each factor iteratively until convergence (root mean square error $<10^{-6}$) was achieved. The likelihood ratios between two models were analyzed with the likelihood ratio test under the χ^2 distribution with a degree of freedom equal to the number of additional factors introduced by the model with the greater number of variables. We also performed additional factor analysis through factoring out for each of the factors of interest and determining if the mitigation by drug B was still significant.

Cell biological network analysis—The overlap between the Gene Ontology term-based pathophysiology-related subnetwork and the targets of drugs that modified rosiglitazone-associated MI reporting odds ratio was evaluated with Fisher's exact test. See Supplementary Methods for details.

Animal experiments—Point-wise comparisons (for example, PAI-1 levels at the end of the 10-week study) were evaluated with an unpaired two-tailed *t* test. Blood glucose and PAI-1 levels were examined such that the average of all groups at day 0 is normalized to that of control by adding the average of control at day 0 and subtracting the average of the respective group at day 0. Thromboelastography (TEG) clotting parameters were evaluated with a one-tailed paired *t* test on the basis of the specific TEG run. One-tailed *t* test was used because our hypothesis was that exenatide would increase CT and CFT while decreasing clot firmness. Each TEG run was made up of a single animal from all of the four independent groups (control, rosiglitazone alone, exenatide alone, and rosiglitazone + exenatide) to account for batch effect variability. All statistical analyses for experimental data were performed in GraphPad Prism 5. $P < 0.05$ was deemed to be significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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B

Drug B	Rosiglitazone		Odds ratio	95% CI	P
	MI without drug B (%)	MI with drug B (%)			
Exenatide	19,910/58,604 (33.97)	95/4,460 (2.13)	0.04	(0.03-0.05)	7.0E-203
Metformin	17,481/51,266 (34.10)	2,524/11,798 (21.39)	0.53	(0.50-0.55)	6.0E-154
Aspirin	19,107/57,813 (33.05)	898/5,251 (17.10)	0.42	(0.39-0.45)	1.8E-118
Furosemide	19,439/59,555 (32.64)	566/3,509 (16.13)	0.40	(0.36-0.43)	4.5E-87
Acetaminophen	19,623/60,635 (32.36)	382/2,429 (15.73)	0.39	(0.35-0.44)	1.5E-62
Levothyroxine	19,591/60,660 (32.30)	414/2,404 (17.22)	0.44	(0.39-0.49)	5.8E-52
Warfarin	19,824/61,666 (32.15)	181/1,398 (12.95)	0.31	(0.27-0.37)	2.2E-47
Glibenclamide	19,117/59,052 (32.37)	888/4,012 (22.13)	0.59	(0.55-0.64)	1.1E-40
Quetiapine	19,954/62,368 (31.99)	51/696 (7.33)	0.17	(0.13-0.22)	1.9E-34
Gliclazide	19,971/62,409 (32.00)	34/655 (5.19)	0.12	(0.08-0.16)	3.1E-34

Fig. 1. Study design flow chart and effect of various drugs on rosiglitazone-associated MI reports in FAERS

(A) Flow chart of study design. Pink boxes represent the databases used; light blue boxes denote steps implemented in this study; tan box proposes a possible approach that may be taken for clinical validation. (B) Top 10 drugs that alleviated percentage of rosiglitazone-associated MI reports in FAERS, ranked according to their significance. Numbers represent reports of MI divided by the number of non-MI adverse event reports. Significance of odds ratios was evaluated under a log-normal distribution as described in Materials and Methods.

Fig. 2. Clinical databases show an increase in MI reporting in patients using rosiglitazone that is reduced when exenatide is coprescribed

(A) Flow chart for determination of MI odds ratios categorized for disease (diabetes) and drug usage as listed below. Numbers in parentheses represent number of reports. Roman numerals in blue boxes are labels for the data shown below in (B) and (C). (B) MI odds in the entire FAERS for three categories of patients: patients prescribed rosiglitazone (I), patients prescribed metformin (III), and patients with diabetes (V), as well as MI odds in the MSDW database. (C) MI odds ratios when exenatide was prescribed versus when exenatide was not prescribed for patients on rosiglitazone (II), patients on metformin (IV), and patients with diabetes (VI), as well as MI odds in the MSDW database for patients on rosiglitazone. P values were determined by evaluating the odds ratios under a log-normal distribution.

Fig. 3. Relationship of clotting regulation subnetwork to rosiglitazone

A subnetwork was constructed with transcriptional relationships and direct protein-protein interactions to connect PPAR γ to nodes that are described by the Gene Ontology term “regulation of blood coagulation.” Targets of drugs within this subnetwork that modulate rate of reporting of MI in FAERS were identified. Purple indicates rosiglitazone, yellow indicates PPAR γ , green indicates drugs from FAERS that significantly reduced MI adverse event reports from rosiglitazone-treated individuals based on the odds ratio’s CI evaluated under a log-normal distribution as described in Materials and Methods, blue indicates the corresponding drug targets for green boxes, and pink indicates intermediate gene products from the human interactome. Genes are in *italics*, and gene products (proteins) and drugs are in normal case. Please note that not all edges are direct.

Fig. 4. Physiological and biochemical parameters in wild-type and *db/db* mice treated with rosiglitazone and/or exenatide

The effects of saline control (CON), rosiglitazone (ROS), exenatide (EXT), or rosiglitazone + exenatide combination (R + E) on physiological and biochemical parameters were measured. **(A)** Effect of drug treatment on PAI-1 levels, body weight, and plasma glucose in B6 (wild-type) mice. Data are means $\pm$ SD ($n = 4$ mice per group) for PAI-1 levels and plasma glucose. Data are means for body weight ($n = 4$ mice per group). **(B)** Effect of varying drug treatment on PAI-1 levels, body weight, and plasma glucose in *db/db* mice. Data are means $\pm$ SD (control: 4 mice; rosiglitazone: 14 mice; exenatide: 4 mice; rosiglitazone + exenatide: 15 mice) for PAI-1 levels and plasma glucose. Data are means for body weight ($n = 4$ mice per group). Statistical significance for plasma glucose was determined by a two-tailed unpaired t test. $*P < 0.01$ between rosiglitazone and rosiglitazone + exenatide with a two-tailed paired t test.

Fig. 5. Effect of drug treatment on different clotting characteristics as measured by thromboelastography and cardiac function as measured by echocardiography

(A) Measurements were done in a paired fashion where one rosiglitazone-treated mouse ($n = 7$) was compared to one rosiglitazone + exenatide-treated mouse ($n = 7$), which were sacrificed within 60 min of the measurements. Three clotting characteristics are shown: CT, CFT, and MCF. These values were compared in a pairwise fashion between rosiglitazone and rosiglitazone + exenatide. P values were determined by one-tailed t test. All combinations of pairwise one-tailed t test values are shown below the respective plots, with significant combinations in bold. (B) Scatter plots for thromboelastography data from all treatment groups. (C) Scatter plots for fractional shortening measured by echocardiography from all treatment groups. Each data point in (B) and (C) represents an individual mouse.

Table 1
Drug combinations that mitigate serious adverse events reported in FAERS

We examined other potentially useful combinations by comparing individual drugs (or classes of drugs) based on therapeutic indication from their anatomic therapeutic code index. These combinations were examined for statistical significance with the methods as described in Materials and Methods. Drug B reduces the percentage of adverse event reports listing adverse event of interest associated with drug A. Data are number of events out of total number of patients on drug A or drug A + drug B. A complete list is provided in table S14; a select subset based on the diversity of the combinations is shown here. SSRI, selective serotonin reuptake inhibitor.

Drug A	Drug B	Adverse event associated with drug A	Adverse event occurrence with drug A, without drug B	Adverse event occurrence with drug A + drug B	95% CI	P
Niacin	Acetaminophen	Flushing	16,878/42,886 (39.4%)	511/2,748 (18.6%)	0.32–0.39	<0.001
Bisphosphonates	Folic acid	Femur fracture	9,584/71,729 (13.4%)	172/7,120 (2.4%)	0.14–0.19	<0.001
Montelukast	Salbutamol	Allergic granulomatous angitis	840/21,970 (3.7%)	96/11,914 (0.8%)	0.17–0.26	<0.001
Statins	Lisinopril	Rhabdomyolysis	8,291/286,119 (2.9%)	582/35,870 (1.6%)	0.51–0.60	<0.001
SSRI	H ₂ antagonist	Completed suicide	7,108/226,090 (3.1%)	64/11,590 (0.6%)	0.13–0.22	<0.001
Propofol	Fentanyl	Anaphylactic shock	428/6,389 (6.3%)	90/3,868 (2.3%)	0.28–0.44	<0.001