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The nasty surprise of a complex drug–drug interaction

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In vitro **investigation of pharmacokinetic drug–drug interactions (DDIs) has officially been part of the regulatory pathway for new drugs in the USA since the publication of an FDA guidance on the subject in 1997. The field has continued to evolve, driven by preclinical and clinical experience, improved understanding of the molecular basis of DDIs, technological advances, and a continuous dialogue between the FDA and pharmaceutical industry scientists. Some striking DDIs involve multiple molecular species and targets; their mechanisms and magnitude would have been difficult or impossible to predict with available *in vitro* tools. This article focuses on one such example.**

Industry perspective

It is no secret that these are tough times for the pharmaceutical industry. Poor productivity, layoffs, late-stage drug failures, more layoffs, patent expirations, the demise of the blockbuster model, restructuring, close scrutiny by Congress, mega-mergers, yet more layoffs, and so on. There are so many ways that drugs can fail: most candidates never make it to the clinic... if they get to the clinic, they might fail to perform adequately in clinical trials... or they can survive clinical trials yet fail to win approval by the FDA or other regulatory bodies. A drug might simply not be as effective as anticipated; poor pharmacokinetic (PK; Box 1) properties might prevent it from reaching its intended target in therapeutic doses, or it might be proven unsafe.

As if those hurdles were not enough, a sponsor has to worry that a new drug might interact with other drugs that are co-administered with its potential blockbuster, which could result in serious side-effects. Both the hype and the risk are fueled by direct-to-consumer advertising, which in the author's opinion is part of the problem with regard to drug–drug interactions (DDIs; Box 1).

Late-stage drug failures are costly – wasting precious time, manpower and hundreds of millions of dollars – and have contributed to the declining overall productivity in the industry in recent years. Thus, the mantra 'fail fast, fail cheap' has led to more and more resources being applied earlier in the drug development process to weed out problem drugs before they reach clinical testing.

Preclinical testing to avoid DDIs

Over the past 20 years, preclinical evaluation of the absorption, distribution, metabolism and excretion properties and potential PK-based DDIs of new drug candidates has become an integral part of the drug development paradigm.

Pharmaceutical researchers evaluate pathways of drug metabolism using human liver microsomes, hepatocytes and recombinant enzymes. This leads to identification of metabolites (some of which might have safety issues and can be tested separately) and prediction of potential clinical DDIs mediated by inhibition or induction of drug-metabolizing enzymes (Box 1). This area is fairly mature; most or all of the major enzymes have been identified and cloned, and a full complement of enzyme-specific probe substrates and inhibitors is available. A key driver for this type of testing, which has been required by the FDA since 1997 [1], was the case of terfenadine [2]. The simple interaction between terfenadine and cytochrome P450 (CYP) 3A4 inhibitors is illustrated in Figure 1.

More recently, researchers have begun to evaluate interactions of new drug candidates with drug transporters such as P-glycoprotein, a membrane protein that mediates the distribution and excretion of many drugs. The OATP family of transporters (particularly OATP1B1) is also of great interest, owing to the potential

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BOX 1

Explanation of terms

Pharmacokinetics (PK) is the branch of pharmacology that considers what the body does to a drug, as distinguished from pharmacodynamics (PD), which is the study of what a drug does to the body. Although drug–drug interactions (DDIs) can involve either PK or PD, only PK-based DDIs will be considered in this article. Every DDI involves both an interacting 'drug' (a compound – not necessarily a drug; it could be a dietary component or herbal supplement, for example – that causes a change in exposure to another compound) and a 'victim' (a drug whose exposure is altered when it is administered in the presence of an interacting drug).

Throughout this article, the words 'we', 'our' and 'us' refer to the pharmaceutical industry as a whole.

Inhibition of a drug-metabolizing enzyme is a common mechanism of drug interaction, which can lead to a marked increase in exposure to the victim drug. The result can be expected or unexpected side-effects or even death. The opposite effect, enzyme induction (an increase in the amount of enzyme) can lead to a decrease in exposure to (and possible therapeutic failure of) the victim drug.

Interacting drugs can also inhibit or induce drug transporters. Because transporters are expressed throughout the body, it is difficult to predict *a priori* the consequences of such an interaction on exposure to or distribution of a victim drug, and because *in vitro* models and pharmacologic probes are lacking, it is less feasible to test routinely for interactions with transporters.

for DDIs involving uptake into hepatocytes. This field is less mature than *in vitro* drug metabolism and lacks specific pharmacologic probes and definitive, well-accepted assay systems. Testing for interactions with drug transporters (Box 1) was addressed in a 2006 update [3] (still in draft form at the time of publication of this article) of the 1997 FDA metabolism guidance [1].

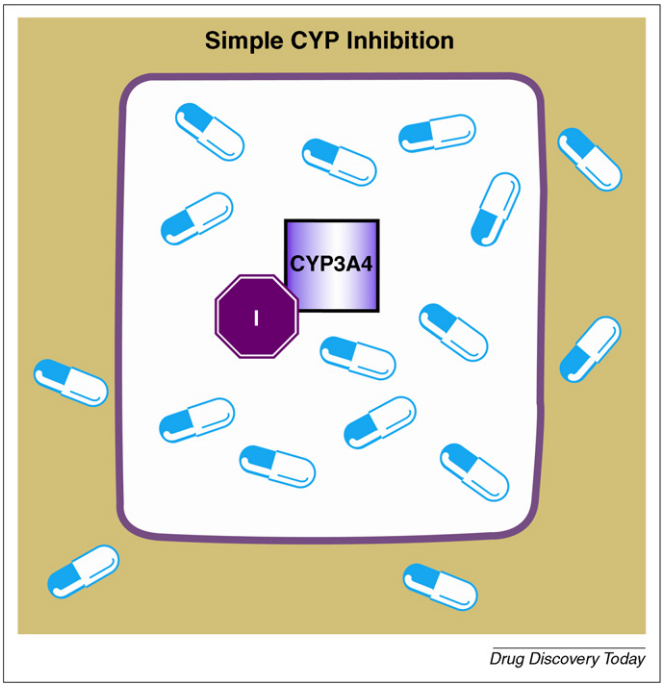

FIGURE 1

Illustration of a simple DDI in a hepatocyte. A CYP3A4 inhibitor (I) such as ketoconazole causes the concentration of a CYP3A4 substrate (blue and white capsules) such as terfenadine to increase because metabolism is blocked.

In vitro testing for drug absorption has developed to the point that, in some cases, *in vitro* testing can replace clinical testing. This is the application of the Biopharmaceutics Classification System, a regulatory framework [4] that makes highly soluble and highly permeable active pharmaceutical ingredients in a rapidly dissolving formulation eligible for 'biowaivers'. Such drugs can forgo clinical bioequivalence testing of new formulations, generic equivalents, and so on. Although *in vitro* prediction of absorption is not required by regulatory agencies, it has become a routine practice within the industry, largely driven by the validated predictive power of models such as the Caco-2 cell monolayer [5].

So with all of these tools at our disposal, where do we (Box 1) stand in terms of our ability to predict PK-based DDIs? For the most part, we do a pretty good job in terms of qualitative prediction of DDI potential (i.e. identifying drug combinations in which there is a risk of a clinically meaningful alteration in exposure). We understand most of the important factors – for example, for inhibition of CYP enzymes, that would include K_i (potency for inhibition by the interacting drug), the ratio between the concentration of inhibitor and potency ($[I]/K_i$), fm_{CYP} (the fraction of the victim drug metabolized by the enzyme inhibited by the interacting drug), the mechanism of inhibition (reversible vs. irreversible or time-dependent), PK of the interacting drug, and the dosing interval between the interacting drug and the victim. Of these, the one factor that we do not have a good handle on is [I], the unbound concentration of the inhibitor at the enzyme (i.e. inside the liver cell). The surrogate parameter, the plasma concentration of the inhibitor, might have little resemblance to the hepatic intracellular concentration because of the presence of uptake transporters (e.g. the OATP family) that can concentrate drugs inside the cells and efflux transporters (e.g. the MRP family) that can pump drugs out of cells. Largely as a result of that knowledge deficit, the quantitative prediction of the magnitude of a given clinical DDI remains problematic in most cases [6].

Regulatory perspective

Nevertheless, the FDA and other regulatory agencies expect to see *in vitro* data on the potential for a new drug candidate to be involved in DDIs, either as an interacting drug or as a victim. This is enabled by approximately 20 years of advances in our understanding of PKs at the molecular level, in turn driven by the pain of several drug withdrawals because of metabolism-based DDIs (Table 1). Of course, there have been many other drugs withdrawn from the market for other reasons; these are some that were withdrawn, at least in part, because of DDIs.

Given the extent of our knowledge of the mechanisms of DDIs, the amount of preclinical testing that drug sponsors perform to weed out bad actors before they reach the clinic and the years of clinical trials required before a new drug reaches the market, it is remarkable that we continue to be surprised by some clinical DDIs. But maybe it should not be so surprising; the total number of people involved in clinical trials, particularly the subset of trials in which drug exposure is closely monitored, is a drop in the bucket compared with the millions of prescriptions that are written every year for a blockbuster drug when it is finally commercialized.

TABLE 1

Drugs withdrawn from the market because of DDIs.

U.S. brand name	Generic name	Victim or interacting drug	Problem partners	Result of DDI
Baycol	Cerivastatin	Victim	Gemfibrozil	Rhabdomyolysis
Hismanal	Astemizole	Victim	CYP3A4 inhibitors	Severe cardiac arrhythmia
Posicor	Mibrefadil	Interacting	Astemizole, cisapride, terfenadine	Severe cardiac arrhythmia
			Lovastatin, simvastatin	Rhabdomyolysis
Propulsid	Cisapride	Victim	CYP3A4 inhibitors	Severe cardiac arrhythmia
Seldane	Terfenadine	Victim	CYP3A4 inhibitors	Severe cardiac arrhythmia

When a potential DDI is identified through *in vitro* testing, clinical trials can be designed specifically to detect and quantify the magnitude of the interaction by monitoring the exposure (AUC, or area under the curve of plasma concentration vs. time) of the putative victim drug. The consequences of a clinical DDI can range from no change in clinical practice for a victim drug with a wide therapeutic ratio, to dose adjustment for a victim drug with a narrow therapeutic range or whose exposure is increased greatly when co-administered with an interacting drug, to withdrawal from the market in cases in which dose adjustment is inadequate to prevent the deaths of patients. The most severe consequences tend to apply to drugs that are metabolized, wholly or mostly (>80% is a good benchmark [6]), by a single liver enzyme because if that enzyme is inhibited by an interacting drug, the body might have no other means of eliminating it.

Complexity and predictability

As stated earlier, enough is known to identify the potential for metabolism-based DDIs preclinically, even though we might not be able to predict their magnitude. In a nutshell, the testing process goes something like this:

New drug as victim

- Using human liver microsomes (the rationale being that most drugs are broken down by liver microsomal enzymes) and specific chemical inhibitors, identify the enzyme or enzymes that metabolize(s) a new drug candidate.
- Particularly if the new drug is metabolized by a single pathway, eventually design a clinical trial (if it gets to that point in development) to assess the extent of the increase in exposure to the new drug upon co-administration of an inhibitor or inducer of its metabolism.

New drug as interacting drug

- Using human liver microsomes and enzyme-specific probe substrates, test for inhibition of several CYPs by the new drug.
- Using human hepatocytes, test for induction of several CYPs by the new drug.
- If the new drug's potency for enzyme inhibition or induction (if any) is similar to its circulating concentration in the plasma (which is not known until after Phase I), design a clinical trial to assess the impact of co-administration of the new drug on exposure to a drug that is known to be metabolized by that pathway. Sensitive substrates for different CYPs and transporters are published by the FDA [3].

What if a drug interacted with more than one enzyme or with an enzyme and a transporter? It is possible to test for interactions with one target at a time, so it should be feasible to detect multiple potential interactions if the right targets are tested.

What if a metabolite of one drug interacted with other drugs? Despite our best efforts, we might not know about it until some time after it had been on the market and caused problems in enough patients that a pattern became evident. The rest of this article focuses on one such case.

Repaglinide, gemfibrozil and itraconazole

The case involves three players: repaglinide, gemfibrozil and itraconazole. Repaglinide is a short-acting oral hypoglycemic drug developed to lower blood glucose in patients with Type 2 diabetes [7]. More precisely, it is designed to be taken before a meal to prevent postprandial hyperglycemia. It is completely metabolized, and CYP2C8 and CYP3A4 have been identified as the enzymes responsible for its elimination [8]. *In vitro*, CYP2C8 and CYP3A4 metabolize therapeutic concentrations of repaglinide at similar rates [9]. Drugs in the fibrate class, including gemfibrozil, are widely used in the treatment of the dyslipidemia associated with Type 2 diabetes and the prediabetic state known as metabolic syndrome, which includes obesity, hypertension, hyperglycemia, hypercholesterolemia and hypertriglyceridemia. As a result, repaglinide and gemfibrozil are often co-prescribed. Many marketed drugs are inhibitors of CYP3A4, including the antifungal itraconazole.

In clinical studies [10], the AUC of repaglinide was 8.1-fold higher in the presence than in the absence of gemfibrozil and its elimination half-life ($t_{1/2}$) was prolonged from 1.3 to 3.7 h. Itraconazole alone increased the repaglinide AUC only 40%. Surprisingly, when co-administered with the combination of gemfibrozil and itraconazole, the AUC of repaglinide was 19.4-fold higher than in their absence and the $t_{1/2}$ of repaglinide was 6.1 h. At 7 h, the plasma concentration of repaglinide was 28.6-fold higher in the presence of gemfibrozil and 70.4-fold higher in the presence of the gemfibrozil–itraconazole combination. In addition to these PK effects, gemfibrozil alone, and to an even greater extent in combination with itraconazole, considerably enhanced and prolonged the therapeutic effect of repaglinide. As a result, it became a long-acting and more powerful hypoglycemic agent.

As a point of reference, it should be noted that a twofold increase in AUC is generally considered clinically significant, and for some drugs with a narrow therapeutic ratio (e.g. digoxin), even a 50% increase in AUC can be important.

As if the short-term consequence of low blood sugar were not enough of a problem, the long-term effects might be even worse.

According to a recent, large retrospective study of older diabetics (mean age 65 years), one or more episodes of hypoglycemia serious enough to warrant hospitalization or a trip to the emergency room is associated with an increased risk of later diagnosis with dementia [11].

So how to explain the clinical findings? *In vitro*, gemfibrozil is a moderate inhibitor of CYP2C8 and does not inhibit CYP3A4 at clinically relevant concentrations [9]. When gemfibrozil is taken up by hepatocytes, however, it is rapidly conjugated with glucuronic acid to form gemfibrozil-1-O-β-glucuronide, which is a potent inhibitor of both CYP2C8 and the hepatic uptake transporter, OATP1B1 [12]. There is no direct *in vitro* evidence that repaglinide is taken up into hepatocytes by OATP1B1; however, clinical pharmacogenetic studies indicate that the PK of repaglinide is dependent on expression of functional variants of the SLCO1B1 gene, which codes for OATP1B1 [13,14]. Clinical DDI studies lead to the same conclusion [14,15].

If one accepts that repaglinide is a substrate of OATP1B1, as well as CYP2C8, what should one conclude about the involvement of these two molecular targets in the gemfibrozil–repaglinide DDI? It is tempting to invoke the involvement of OATP1B1, particularly given its involvement in other notorious DDIs (such as the one between gemfibrozil and cerivastatin [16] that led, in part, to withdrawal of the latter from the market). However, several clinical observations suggest, indirectly, that this is not the case [12,14], leaving us with inhibition of CYP2C8 as the cause of the gemfibrozil–repaglinide DDI. The fact that the lack of involvement of OATP1B1 cannot be proven directly underscores the need for more specific, more definitive assay systems and pharmacologic reagents for transporters. As an example, cyclosporine A, a probe used both *in vitro* and *in vivo*, inhibits uptake via OATP1B1, efflux via P-glycoprotein and metabolism via CYP3A4 [17–20], making it nearly impossible to draw definitive pharmacologic conclusions.

Why is repaglinide so exquisitely sensitive to inhibition of CYP2C8 by gemfibrozil-1-O-β-glucuronide? Fortunately, an eightfold higher AUC of a victim drug in the presence of a CYP inhibitor is rare, particularly when another CYP (3A4 in this case) also metabolizes the victim drug *in vitro*. The surprising magnitude of the increase in exposure was explained by the report that gemfibrozil-1-O-β-glucuronide is a metabolism-dependent, essentially irreversible, inhibitor of CYP2C8 *in vitro* [21]. The inhibitory species (an oxidation product of gemfibrozil-1-O-β-glucuronide) and site of reaction on the enzyme (heme prosthetic group) have been identified recently [22]. As a result, the impact on the PK of a victim drug that is a CYP2C8 substrate, such as repaglinide, is exaggerated.

Given the modest effect of the CYP3A4 inhibitor, itraconazole, on the AUC of repaglinide, why does it have a much greater impact when administered in the presence of gemfibrozil? This can be accounted for if CYP3A4 is a minor metabolic pathway for repaglinide *in vivo* at low therapeutic concentrations of the drug [9,10], only playing a role at high concentrations. In that case, a CYP3A4 inhibitor such as itraconazole would only increase the AUC of repaglinide if the major metabolic pathway, CYP2C8, were inhibited, as in the presence of gemfibrozil. This is an example of a potent inhibitor of one CYP shifting the fm_{CYP} balance toward a different CYP. The complex nature of this DDI is illustrated in Figures 2 and 3.

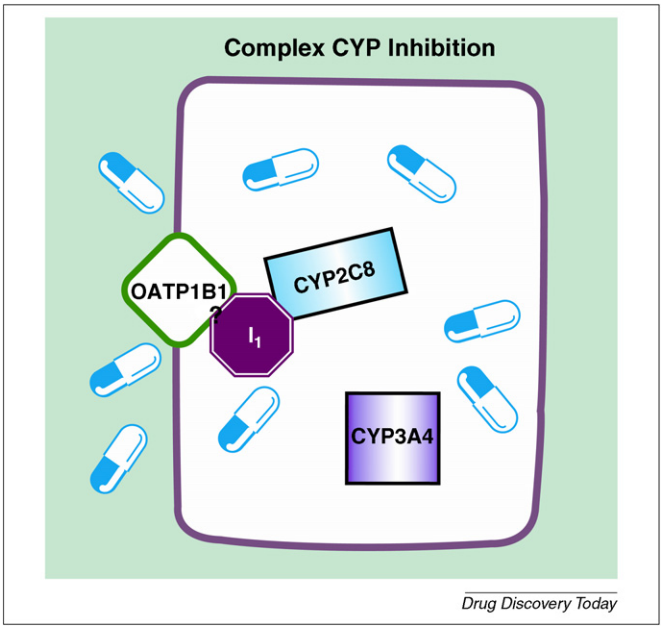

FIGURE 2

The beginning of a complex DDI. A CYP2C8 inhibitor such as gemfibrozil glucuronide (I_1; generated from gemfibrozil inside the hepatocyte) causes an increase in the concentration of a CYP2C8 substrate (blue and white capsules) such as repaglinide because metabolism is blocked at low concentrations of the substrate. CYP3A4 is active at higher concentrations, preventing repaglinide levels from getting too high. The uptake transporter, OATP1B1, is shown because it is also inhibited by gemfibrozil glucuronide. Inhibition of repaglinide uptake might have no involvement in the DDI with gemfibrozil, but that is very difficult to prove.

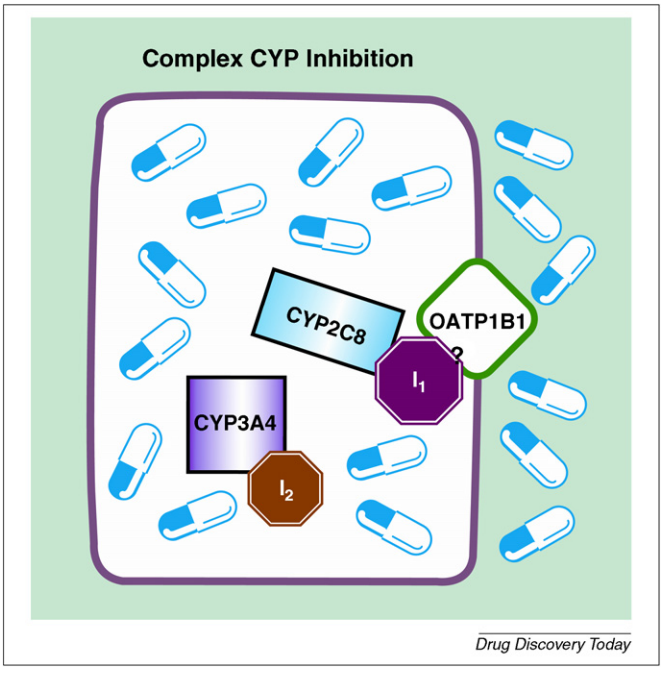

FIGURE 3

A full-blown complex DDI. Addition of a CYP3A4 inhibitor (I_2) such as itraconazole (which by itself has little effect) on top of gemfibrozil causes repaglinide levels to increase substantially.

Lessons learned

Could this DDI have been predicted by *in vitro* testing? Should it have been caught before patients were put at risk? What can be done to do a better job of predicting such DDIs in the future?

This DDI could certainly have been picked up by *in vitro* testing before the drugs reached the clinic, but the required assay protocol was not and still is not in routine use. As Ogilvie *et al.* [21] suggest, a generic *in vitro* screen would involve two steps: incubation of a test compound with alamethicin-treated human liver microsomes and uridine diphosphate glucuronic acid to form the glucuronide, followed by incubation with NADPH and a probe substrate(s) to assess inhibition of one or more CYPs. An additional step (with appropriate controls) could be used to distinguish between time-dependent and reversible inhibitors. The situation is completely analogous to testing for CYP inhibition; probably every pharmaceutical company now routinely tests new drug candidates for both reversible and time-dependent inhibition of one or more CYPs [23]. In the case of glucuronides, it would be prudent to, first, test for formation of a glucuronide, then test for inhibition of CYPs as outlined above.

It is unreasonable to expect that this DDI should have been detected preclinically. As Baer *et al.* [22] note, there were no obvious structural alerts in gemfibrozil-1-O-β-glucuronide other than the fact that it is an acyl glucuronide, a structural feature known to predict potential tissue reactivity and toxicity [24] but not time-dependent CYP inhibition. Fortunately, now that the DDI is known, patients can be switched from gemfibrozil to a different drug in the same class, such as fenofibrate or bezafibrate, neither of which has a PK interaction with repaglinide.

In the future, we will almost certainly get better at catching DDIs such as this before a new drug obtains regulatory approval. This will be driven, in part, by the development of more definitive *in vitro* assay systems, particularly for drug transporters, such as OATP1B1, and more specific pharmacologic reagents. Will we catch all of them? Almost certainly not. Should we try to catch all of them, or proceed with the knowledge that one is going to slip by occasionally? That is a question for high-level risk–benefit–cost analysis within the pharmaceutical industry, with input from regulatory agencies. In general, these agencies do their best to avoid false negatives.

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