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Research Article

Drug-Drug Combinations Can Enhance Toxicity as Shown by Monocyte-Derived Hepatocyte-like Cells From Patients With Idiosyncratic Drug-Induced Liver Injury

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The authors certify that all research involving human subjects was done under full compliance with all government policies and the Helsinki Declaration.

ABSTRACT

Drug-induced liver injury (DILI) is a major cause for acute liver failure and regulatory actions on novel drugs. Individual patient characteristics are the main determinant of idiosyncratic DILI, making idiosyncratic DILI (iDILI) one of the most challenging diagnoses in hepatology. Individual drug-drug interactions might play a role in iDILI. However, the current approaches to iDILI diagnosis are focused on single drugs as causative agents. For the present analysis, 48 patients with acute liver injury who took 2 drugs and who were diagnosed as iDILI were investigated. A novel *in vitro* test was employed using monocyte-derived hepatocyte-like cells (MH cells) generated from these patients. iDILI diagnosis and causality were evaluated using clinical causality assessment supported by Roussel-Uclaf Causality Assessment Method. In 13 of these 48 patients (27%), combinations of drugs increased toxicity in the MH test when compared with the single drugs. Interestingly, whereas in 24 cases (50%) drug-drug combinations did not enhance toxicity, in 11 cases (23%) only the combinations caused toxicity. The incidence of severe cases fulfilling Hy's law was higher in patients with positive interactions (57% vs 43%; $p = .04$), with acute liver failure occurring in 40% versus 8% ($p = .01$). The most common drug combinations causing increased toxicity were amoxicillin/clavulanate (8 of 9 cases) and diclofenac in combination with steroid hormones (4 of 9 cases). Drug-drug interactions may influence the incidence and/or the severity of idiosyncratic DILI. MH cell testing can identify relevant drug-drug interactions. The data generated by this approach may improve patient safety.

Study identifier: ClinicalTrials.gov NCT 02353455.

Key words: acute liver injury; DILI; toxicity; medication; amoxicillin; clavulanate; diclofenac; herb-drug interactions.

INTRODUCTION

Drug-induced liver injury (DILI) is an adverse drug reaction that is the result of an overdose (eg, acetaminophen) (Ramachandran and Jaeschke, 2018) or of an idiosyncratic reaction (Kullak-Ublick *et al.*, 2017). Idiosyncratic DILI (iDILI) has no obvious dose relationship and its occurrence is influenced by multiple factors, such as age, gender, genetics, underlying diseases, environmental factors, and comedications (Chen *et al.*, 2015; Fontana, 2014; Ortega-Alonso *et al.*, 2016).

The pathogenesis of iDILI is complex, yet most of the current models imply a "first hit" by eg, an accumulation of drug metabolites that lead to hepatocyte damage by reactive metabolites (Jaeschke *et al.*, 2012). Following the initial injury, cell death and immune activation lead to continuing or even increasing liver damage that finally results in clinically significant iDILI or acute

liver failure (ALF). However, even in iDILI drug dose may be important: This is supported by the observation of a higher iDILI risk with drugs that are used at higher daily doses (Chen *et al.*, 2013). Furthermore, metabolism by hepatic CYP450 enzymes seems also to be a risk factor for iDILI (Yu *et al.*, 2014). The gender difference in CYP450 activity (Wolbold *et al.*, 2003) may cause the predominance of female iDILI patients when drugs like diclofenac, tetracyclines, and nitrofurantoin are the cause of iDILI (Ortega-Alonso *et al.*, 2016). Increased susceptibility initiating iDILI may be caused by a left shift of the individual dose-response curve for liver injury (Roth and Ganey, 2010), which might be due to genetic or environmental factors, comorbidities, or comedications. This increased susceptibility leads to liver damage followed by complex events involving cell death signaling, mitochondrial damage, and immune activation (Iorga *et al.*, 2017; Ramachandran *et al.*, 2018).

At present, iDILI is a diagnosis of exclusion, necessitating comprehensive investigations to rule out other, more common causes of liver injury (Chalasani *et al.*, 2014; Kullak-Ublick *et al.*, 2017). Despite the ongoing investigation of possible biochemical markers (Mcgill and Jaeschke, 2014), iDILI diagnosis is still based on clinical judgment. Thus, the diagnosis of iDILI and moreover the assessment of drug causality are a major challenge, particularly when the intake of several drugs coincides with the iDILI event (Anderson and Borlak, 2011; Björnsson and Hoofnagle, 2016). Current causality assessment methods rely on drug history, causality scores such as Roussel-Uclaf Causality Assessment Method and expert opinion (Chalasani *et al.*, 2014; Scalfaro *et al.*, 2017). This requires a complex and comprehensive data collection to inform causality adjudication (Avigan *et al.*, 2014), which is not always possible in cases outside clinical trials. At present, causality assessment focuses on a single drug as cause for iDILI by assessing the temporal relationship of drug exposure to the suspected iDILI event and ranking the probability of a given drug as culprit by comparing the pattern of liver injury, latency, and course to previously reported data (Chalasani *et al.*, 2014). However, polymedication is frequent in iDILI and may render the correct assessment of causality impossible: Incidence of cases with more than one suspected drug ranges from 4% in a cohort of patients with ALF (Russo *et al.*, 2004) up to 36% of cases (Fontana *et al.*, 2014). In a single center study in 42% of cases, more than one drug was suspected to be causative for iDILI (Idilman *et al.*, 2010). A recent study of iDILI caused by amoxicillin/clavulanate reports a proportion of 77% of patients with 3 or more comedications (DeLemos *et al.*, 2016).

Not much is known about the role of drug-drug interactions (DDIs) in idiosyncratic DILI, although comedications can influence the individual drug-response by altered absorption, distribution, metabolism, and excretion (Palleria *et al.*, 2013). Based on the frequency of liver safety reports to pharmacovigilance databases, a relevant impact of comedications on hepatic safety is suggested (Suzuki *et al.*, 2015). In the context of iDILI, differences in hepatic metabolism do not uniformly imply major changes in overall pharmacokinetic parameters, eg, in the case of diclofenac: The first step in diclofenac metabolism in humans can be 4′-hydroxylation by cytochrome P450 2C9, 5′-hydroxylation by CYP3A4, or glucuronidation by UGT 2B7 (Boelsterli, 2003). The individual contribution of each pathway determines both the quality and quantity of potential reactive metabolites that may trigger an iDILI episode, without necessarily affecting diclofenac plasma levels. Thus, in patients with increased susceptibility for iDILI, DDIs might add to the risk of developing relevant liver injury.

We previously have reported a method to generate hepatocyte-like cells with individual donor characteristics from blood monocytes (Monocyte-derived hepatocyte-like cells) (Benesic *et al.*, 2012). MH cells from iDILI patients show increased susceptibility to the respective drugs involved in the iDILI event and can be used to support diagnosis and causality assessment of iDILI as shown by re-exposure (Benesic *et al.*, 2016, 2018). We here investigated the MH cells of 48 iDILI patients with involvement of several drugs or combination drugs and their response to the single agents and their combinations, respectively.

MATERIALS AND METHODS

Patients. MH cells were generated from patients referred to the University Hospital Munich with acute liver injury and recruited for our study with the diagnosis of idiosyncratic DILI (ClinicalTrials.gov: NCT 02353455). For this investigation, MH cell tests from iDILI patients with intake of at least 2 drugs or a combination drug were evaluated. Written informed consent was obtained from each patient. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki and was approved by the ethics committee of the Faculty of Medicine, LMU Munich (project number 55-13). All authors had access to the study data, reviewed, and approved the final manuscript.

For the current analysis, MH cell tests of 48 iDILI patients with intake of 2 drugs involved in the iDILI—episode in whom drugs and their combinations have been tested, were analyzed (Table 2).

iDILI was defined according to consensus criteria (Aithal et al., 2011): (1) alanine aminotransferase (ALT) activity $\geq$5× upper limit of normal (ULN), (2) alkaline phosphatase (ALP) activity $\geq$2× ULN, or (3) ALT $\geq$ 3× ULN and total bilirubin (TB) $\geq$2× ULN. The ULNs for aspartate aminotransferase activity and ALT were 35 U/l for women and 50 U/l for men, the ULN for ALP was 105 U/l for women and 135 U/l for men, and the ULN for TB was 1 mg/dl. The diagnostic workup included virology testing (Hepatitis A-E, CMV, and EBV), liver ultrasound, serologic testing for autoantibodies and liver histology, where available.

Acute liver failure was defined as INR $\geq$ 1.5 and clinical signs of mental alterations.

Hy's law criteria were fulfilled when laboratory testing showed ALT $\geq$ 3× ULN, bilirubin $\geq$ 2× ULN, and AP $<$ 2× ULN.

MH cell testing was performed after blood sampling and generation of MH cells as described earlier (Benesic *et al.*, 2012). Patient-derived MH cells were seeded in 96-well plates and exposed to the respective drugs and their combinations for 48 h at concentrations of 1× Cmax and 10× Cmax, as previously published (Benesic *et al.*, 2016, 2018). Each data point was measured in triplicate. The CytoTox96 nonradioactive assay (Promega, Mannheim, Germany) was used for determination of lactate dehydrogenase (LDH) in cell culture supernatants and cell lysates. From LDH in supernatants and lysates, release of LDH was calculated as percentage of LDH in supernatant relative to total LDH (supernatant + lysate). Toxicity was calculated as percentage of LDH release minus LDH release with vehicle control divided by the difference of LDH release after complete lysis using 1% TWEEN 100 and vehicle control. In order to compensate for variance in seeding density, toxicity values were divided by 2× standard deviation (ULN) of the individual control wells. A test result was considered positive with a signal of $\geq$2× ULN as previously published (Benesic *et al.*, 2016, 2018).

The effects of the drug-drug combinations were compared with the effects of the single compounds on MH cells and classified as "no interaction," when the effect of the combination did

Table 1. Results From Drug-Drug Combination Testing in MH Cells of Healthy Tolerators (Healthy) and Patients With Other Acute Liver Injury (Other ALI)

	Controls		
	Total	Healthy/ Other ALI	MH Result Median (Range)
All combinations tested	138	84/54	0.16 (0–1.93)
Combinations taken by patients	26	3/23	0.13 (0–1.63)
Amoxicillin + clavulanate	58	31/27	0.35 (0–1.93)
Diclofenac + steroid hormone	33	24/9	0.31 (0–0.84)

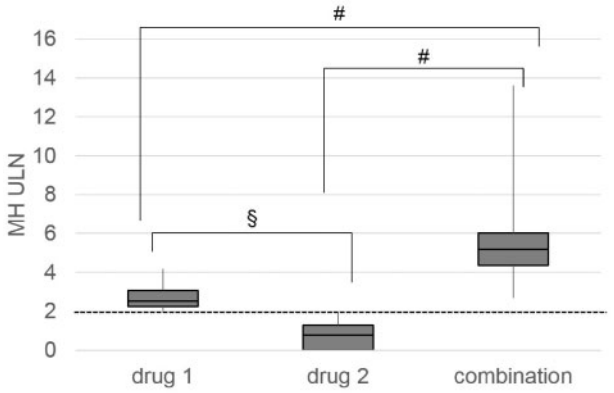

Figure 1. MH cell test results from iDILI patients showing an additive effect of drug 1 and drug 2 in the MH cell signal. Dotted line: cutoff for positive MH cell test. $^{\#}p < .016$ versus combination. $^{\S}p < .016$ drug 1 versus drug 2.

not exceed the effect of the positive drug. If one or both of the single drugs were tested MH positive and the combination evoked an MH signal that was higher than the signal of the positive drug, the effect was termed "additive." If there was no effect of both drugs alone (MH ULN < 2 with drugs alone), but the combination caused a significant toxic response (MH ULN ≥ 2) the effect was termed "synergistic" (Cedergreen, 2014; Hinder, 2011).

The drugs and combinations presented were also tested in a cohort of MH cell donors that did not suffer from iDILI by the respective drugs (total N = 138). In MH cells of these control donors, no relevant signals were observed (Table 1). Data presented represent the results of 10× Cmax values.

Statistical analysis. Data were analyzed using SPSS software (IBM, Armonk, New York, version 25.0.0.1). After testing for a normal distribution, parametric or nonparametric tests (chi-square test, Fisher's exact test, Student's *t* test, Kruskal-Wallis test, or Mann-Whitney *U* test) were applied. $p < .05$ was considered to indicate a statically significant difference. For data with multiple comparisons (Figs. 1 and 2), the Bonferroni correction was used to define significance. Thus, results were considered to be significantly different at $p < .016$ (3 comparisons: drug 1 vs drug 2, drug 1 vs combination, drug 2 vs combination; resulting in 0.05 divided by 3 = 0.0167).

RESULTS

Patient Characteristics

Of the 48 iDILI patients included in this study, in MH cells of 11 patients a positive signal was only observed when the drug-drug combination was used (synergistic effect). In MH cells of another 13 patients, the signal of the positive drug was increased when compared with the effect of the drug alone (additive). In MH cells of the remaining 24 patients, the drug combination did not elicit a higher signal than the positive drug alone (no interaction). Patient characteristics of the 24 cases with positive interactions (synergistic and additive) compared with the 24 cases without interactions are shown in Table 2. There was no significant difference in age or gender between both groups. The frequency of cases fulfilling Hy's law was significantly higher in the patients with positive interactions ($p = .04$). There was also a higher frequency of ALF in the patients with interaction ($p = .01$). Patients with ALF were exposed to the following combinations: fluspirilen and citalopram (liver transplantation), pirfenidone and esomeprazole (death, see also Benesic *et al.*, 2019) , diclofenac and ethinylestradiol (liver transplantation), diclofenac and estradiol/dienogest (death), carbamazepine and doxycycline (recovered), minocycline and tinidazol (recovered), metamizole and ethinylestradiol

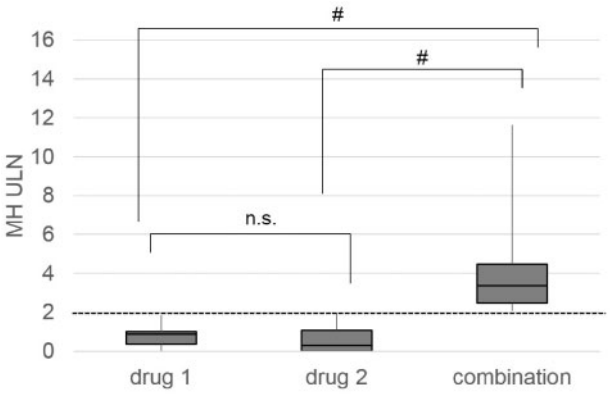

Figure 2. MH cell test results from iDILI patients showing synergistic effects of drug 1 and drug 2 in the MH cell signal. Dotted line: cutoff for positive MH cell test. $^{\#}p < .016$ versus combination. n.s.: not significant drug 1 versus drug 2.

Table 2. Characteristics of Cases With and Without Interaction (Additive and Synergistic)

	DILI Patients		
	Interaction		
	Enhanced Toxicity	No Enhanced Toxicity	*p*
Gender (female/male)	12/12	15/9	.38
Age (median/range)	52.5 (22–75)	53.5 (15–84)	.67
Pattern (HC/MC)	20/4	18/6	.48
ALT max (ULN median/range)	25.7 (1.0–108.2)	36.9 (1.2–121)	.21
AP max (ULN median/ range)	1.8 (0.6–2.8)	1.4 (0.5 + 19.4)	.39
Bilirubin max (ULN median/range)	2.2 (0.5–23.6)	4.6 (0.5–35.1)	.52
Hy's law (±)	14/10	7/17	.04*
Liver failure (±)	10/14	2/22	.01*
Death or transplantation (±)	4/20	2/22	.38

Abbreviations: HC, hepatocellular; MC, mixed or cholestatic; ULN, upper limit normal.
*$p < .05$ positive interaction versus no interaction.

(death), sunitinib and metformin (death), atorvastatin and acitretin (recovered), and amoxicillin/clavulanate (recovered).

MH Test Results

The median time from diagnosis of liver injury to blood sampling was 12 days (minimum 1 day and maximum 177 days). Time from onset to liver injury and MH cell sampling, ALT, AP, and TBL levels of the patients did not differ between the groups at the time of sampling (Supplementary Table 1). We have evaluated the results of 2 samples obtained from the same patient (first sample within the first 4 weeks after onset of liver injury and a second sample within 180 days after diagnosis of liver injury) in a different set of 55 patients and found that the results were reproducible for 151 of 155 drugs (97%). This set included 12 of the patients presented here. Results were concordant in 35 of 36 drugs and combinations tested (97%). Thus, the MH cell test result seems not to be dependent on the timing of blood sampling within 6 months after onset of liver injury. Additionally, the test results did not correlate with liver enzyme elevations.

MH cell testing in the 24 patients who did not show additive or synergistic effects of drug combinations showed a median toxicity signal of 2.2× ULN (range 1.6–6.3). The median results for drug 1 were 2.5× ULN (range 2.0–11.1; p vs combination 0.566). Drug 2 evoked a negative signal in all but 1 patient tested (MH cell results was 2.8 in 1 patient with iDILI by isoniazid and rifampicin tested positive for both drugs; p drug 2 vs combination and drug 2 vs drug 1 < 0.001). In the 13 patients with additive MH results, ULN values were 5.2 (2.7–13.6) with the combination, 2.5 (2.0–4.5) for drug 1 and negative for drug 2, respectively. Testing of MH cells from the 11 patients showing synergistic effects yielded ULN values of 3.4 (2.1–11.6) for the combination and negative results for drug 1 and drug 2, respectively. The results of MH cell testing with additive and synergistic results are provided in Figures 1 and 2.

The drugs that most frequently caused interaction in the MH cells were amoxicillin/clavulanate and diclofenac in combination with steroid hormones (estrogen, gestagen, or corticosteroids). In 8 of 9 amoxicillin/clavulanate cases, interaction was detected, 5 of them synergistic with only the combination being positive and additive effects in 3 cases (Figure 3). In the 3 additive cases, amoxicillin alone was positive in 2 cases and clavulanate was positive in 1 case, respectively. The MH cell signal was increased by the combination. In the 1 case without interaction, clavulanate was positive in the MH cell test without further increase in the signal when the combination was tested.

Diclofenac was implicated in 9 cases total and interaction could be seen in MH cells of 4 patients: 1 synergistic case when diclofenac was administered together with medroxyprogesterone. In the 3 cases showing additive effects, diclofenac was used in combination with ethinylestradiol in 1 case and with corticosteroids (dexamethasone in 2 cases). In the remaining 5 cases without evidence for interaction, comedications consisted of perindopril (1 case), losartan (1 case), and pantoprazole (2 cases). The last case was diclofenac in combination with dienogest and estradiol. However, in this case, diclofenac alone caused 100% toxicity, so that interaction could not be conclusively assessed.

The other combinations and the results on interactions are provided in Table 3. In addition to the interactions with drugs commonly associated with iDILI such as amoxicillin/clavulanate and diclofenac, statins were among the involved drugs in 6 patients (atorvastatin in 5 cases and simvastatin in 1 case). Despite the extensive hepatic metabolism of most statins, MH

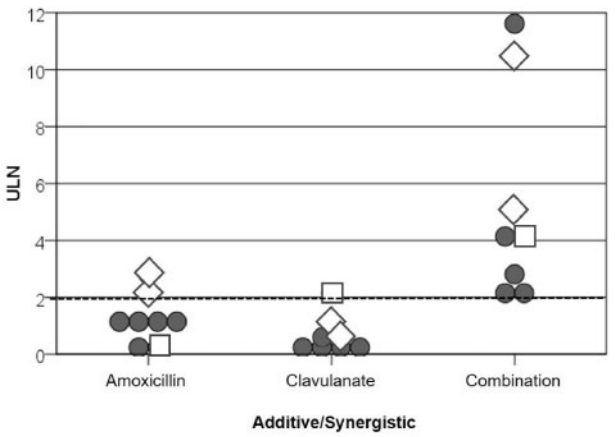

Figure 3. MH cell results with synergistic or additive effects in amoxicillin/clavulanate-iDILI patients (gray circles, synergistic; white diamonds, additive with amoxicillin positive; white squares, additive with clavulanate positive).

Table 3. Drugs and Interactions in MH Cell Test

			Interaction (Enhanced Toxicity)	
Frequency	Drug 1	Drug 2	No	Yes
9	Amoxicillin	Clavulanate	1	8
8	Diclofenac	Dexamethasone		2
		Ethinylestradiol		1
		Medroxyprogesterone		1
		Losartan	1	
		Perindopril	1	
		Valsartan	1	
		Dienogest/estradiol	1	
5	Atorvastatin	Ezetimibe	2	
		Acetylsalicylate	1	
		Pantoprazole	1	
		Acitretin		1
5	Metamizole	Pantoprazole	2	1
		Ethinylestradiol	1	
		Prednisolone	1	
2	Ethinylestradiol	Levonorgestrel	1	
		Dienogest	1	
2	Methylprednisolone	Interferon Beta	1	
		Ethinylestradiol	1	
2	Olanzapine	Sulpiride		1
		Methylprednisolone	1	
1	Alitretinoin	Propofol		1
1	Allopurinol	Ramipril	1	
1	Carbamazepine	Doxycycline		1
1	Clarithromycin	6-Mercaptopurine	1	
1	Clindamycin	Silbinin		1
1	Duloxetine	Prednisolone	1	
1	Estradiol	Levonorgestrel	1	
1	Exemestane	Pantoprazole		1
1	Isoniazid	Rifampicin	1	
1	Fluspirilen	Citalopram		1
1	Minocycline	Tinidazol		1
1	Mirtazapine	Amlodipine	1	
1	Pirfenidone	Esomeprazole		1
1	Simvastatin	Carbimazole		1
1	Sunitinib	Metformin		1

cell testing identified a role of statins in interactions in only 2 cases where statins caused liver injury. Furthermore, proton pump inhibitors (pantoprazole in 2 cases and esomeprazole in 1 case) were involved in 1 additive and 2 synergistic reactions, respectively. In 1 case, we have detected an additive interaction involving herbals/dietary supplements (clindamycin and silybum marianum extract).

DISCUSSION

Despite the frequent occurrence of polymedication in patients with iDILI, little is known about the role of DDIs in the setting of iDILI. A prior publication on amoxicillin/clavulanate-iDILI also suggested increased iDILI severity in the presence of comedications (Yazici *et al.*, 2015). Our results provide evidence that monocyte-derived hepatocyte-like cells (MH cells) from patients with iDILI represent a useful tool to elucidate the impact of potential DDIs on iDILI in the individual patient. Our data show that in half of the investigated patient samples, the MH signal of the culprit drug was not increased by the comedications. However, in the remaining other half of patient samples investigated in this pilot study, the MH cell test showed either additive or synergistic effects. Interestingly, there seems to be a trend toward an increased iDILI severity in patients with enhanced toxicity by DDIs in the MH cell test. This is reflected by a higher frequency of cases fulfilling Hy's law in patients in whom DDIs resulted in increased toxicity in the MH cell test. We also observed a trend toward a higher incidence of ALF in this patient group that reached statistical significance.

The MH cell test results were reproducible within 6 months after onset of liver injury independently of liver enzyme elevations at the time of MH cell sampling. Therefore, the possibility that macrophage activation in ALF leads to enhanced drug combination toxicity in the MH cell test seems unlikely. Most iDILI events are thought to be mediated by adaptive immunity, which is activated by eg, adducts of drugs or their reactive metabolites to proteins, or sublethal cell stress induced by the drugs resulting in the release of danger signals that attract adaptive immune cells (Andrade *et al.*, 2019). This current hypothesis involves an initial trigger that leads to activation of the immune system (Watkins 2019; Williams, 2018). In the setting of the MH cell test, reactions of the patient's cells occur in absence of relevant T-cell or B-cell populations (Benesic *et al.*, 2016). The signal obtained with MH cell testing most likely represents the individual susceptibility to the initial trigger factor (and the strength of this triggering event). Regarding evidence from the literature, immune mechanisms seem to be the main driving force in iDILI progression. Thus, the results provided here show how the individuals susceptibility to iDILI is altered by comedications. The more severe course in the patients with positive interactions in the MH cell test might be due to a more pronounced triggering event that results in a more extensive and prolonged immune activation. Although the potential role of DDIs in iDILI is still controversial, increasing evidence led to inclusion of comedications as a potential risk factor for iDILI in the recently published EASL guidelines (Andrade *et al.*, 2019). In the currently available causality adjudication tools, there is no possibility to adjudicate the potential role of DDI (Tillmann *et al.*, 2019). Recent findings support the potential of amoxicillin/clavulanate to cause clinically significant DDIs (Yazici *et al.*, 2015) with increased potential for severe iDILI. In accordance with previous publications (Hayashi and Fontana, 2014; Ortega-Alonso *et al.*, 2016) the most common combination we found in our patient cohort was amoxicillin/clavulanate. Despite amoxicillin alone has the potential to trigger iDILI events (DeLemos *et al.*, 2016), the combination of both agents seems to confer a greater iDILI risk than amoxicillin alone. This might be due to the fact that both amoxicillin and clavulanate substances undergo extensive hepatic metabolism that might lead to DDIs. Although the combination of amoxicillin and clavulanate reaches the threshold to trigger an iDILI event, amoxicillin alone is below this threshold and adaptation is possible. This might also explain cases in whom rechallenge with amoxicillin alone did not result in recurrence of liver injury after amoxicillin/clavulanate-iDILI. This is supported by our MH cell data: 8 of the 9 amoxicillin/clavulanate-iDILI cases showed interaction. Remarkably, in 6 cases, we observed a synergistic effect of amoxicillin and clavulanate, consistent with the clinical observation that amoxicillin/clavulanate causes iDILI more often than amoxicillin alone. This highlights the ability of the MH cell test to identify relevant DDIs.

Patients with iDILI by diclofenac were also frequent in our cohort. Our data show that combination of diclofenac with either corticosteroids (eg, dexamethasone) or steroid hormones (ethinylestradiol and medroxyprogesterone) increases the MH cell signal evoked by diclofenac. A possible mechanism could be interaction via CYP450 enzymes and UGT2B7 which play a major role in diclofenac metabolism (Boelsterli, 2003). Moreover, there is evidence that oral contraceptives, dexamethasone, and medroxyprogesterone significantly influence both CYP450-metabolism as well as UGT2B7 (Huang *et al.*, 2010; Reimers *et al.*, 2015; Soars *et al.*, 2004). In addition as interaction of these comedications with diclofenac, triggering diclofenac iDILI and/or increasing iDILI severity is supported by the potential role of UGT/CYP-polymorphisms in diclofenac iDILI (Lazarska *et al.*, 2018) and concomitant use of oral contraceptives might be partly responsible for the female predominance in relevant diclofenac iDILI (Ortega-Alonso *et al.*, 2016). The lack of interactions in MH cell tests with drugs that are not known to significantly impact these metabolic pathways (eg, losartan, perindopril) provides additional evidence for specific drug-drug combinations that are capable to increase the iDILI risk of individual patients.

Statins were also present in our MH cell test data set, yet did cause interaction in only 2 of the 7 cases involving statin intake. The respective iDILI patients were medicated with atorvastatin and simvastatin, both known to interact with hepatic CYP450-metabolism (Feidt *et al.*, 2010; Martínez-Gómez *et al.*, 2019). In 3 other cases, we detected MH cell interactions with proton pump inhibitors: esomeprazole with pirfenidone, which reflects current regulatory concerns on possible interaction due to CYP1A2-induction (Yu *et al.*, 2018). Two other cases involve pantoprazole with exemestane and metamizole, respectively. In the case of exemestane/pantoprazole, MH cell test results suggest pantoprazole as the culprit and an additive effect of exemestane, possibly by sharing CYP2C19 metabolism (Meyer, 1996; Peterson *et al.*, 2017; Schwab *et al.*, 2004). In the case of metamizole/pantoprazole, the effect is synergistic and neither pantoprazole nor metamizole alone evoked an MH cell signal. However, given the extensive metabolism of metamizole and its potential risk of immune mediated drug reactions, an interaction triggering or aggravating iDILI is conceivable (García-Martín *et al.*, 2015). In one case, a positive interaction of clindamycin and silybum marianum extract was observed (Benesic and Gerbes, 2018), emphasizing that in the context of iDILI by herbals and dietary supplements their potential to interact with prescription medicines (Awortwe *et al.*, 2018) might by of importance.

In conclusion, our data provide evidence that DDIs in iDILI deserve further investigation of implicated drugs and

comedications in order to identify combinations that pose increased risk on patients. In the case of amoxicillin/clavulanate, the MH cell test shows synergistic toxicity of the combination in 56% of the patients with amoxicillin/clavulanate-iDILI investigated, whereas the single agents were not sufficient to cause significant effects. Thus, the MH cell test is a promising platform to screen potential DDIs in blood samples of iDILI patients. The unique data generated from the MH cell approach in combination with novel computational approaches (Chen *et al.*, 2013; Hammann *et al.*, 2019; Yu *et al.*, 2014) might substantially improve our understanding of idiosyncratic liver injury.

SUPPLEMENTARY DATA

Supplementary data are available at *Toxicological Sciences* online.

DECLARATION OF CONFLICTING INTERESTS

A.B. and A.L.G.: owners of IP and stockholders MetaHeps GmbH. K.J.: nothing to disclose.

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AUTHOR CONTRIBUTIONS

A.B., K.J., and A.L.G. have contributed conception and design of the study, analysis and/or interpretation of data, drafting of the manuscript, and approval of the final version of the manuscript.

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