The influence of drug properties and host factors on delayed onset of symptoms in drug-induced liver injury

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Abbreviations: AC, amoxicillin-clavulanate; BDDCS, biopharmaceutical drug disposition classification system; BSEP, bile salt export pump; CI, confidence interval; DILI, drug-induced liver injury; DOP, delayed onset potential; HLA, human leucocyte antigen; nDOP, no DOP; OR, odds ratio.

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ABSTRACT

Background & Aims: Most patients with drug-induced liver injury (DILI) manifest clinical symptoms while on therapy, while some patients manifest days or weeks after drug cessation (delayed onset). This challenges DILI causality assessment and diagnosis. Factors contributing to the delayed onset phenotype are currently unknown. We explored factors contributing to delayed onset of DILI by analysing culprit drug properties, host factors and their interactions in a large patient population from the Spanish DILI Registry.

Methods: Clinical information from 388 patients (69 presented delayed onset) and drug properties of 43 causative drugs (45 active ingredients) were analysed. A two-tier regression-based model was used to assess host/drug interactions affecting the probability of delayed onset.

Results: Antibacterial and anti-inflammatory drugs accounted for the delayed onset cases. Drug property of <50% hepatic metabolism (odds ratio [OR] 11.06, 95% confidence interval [95% CI]: 4.4-32.2, P = 0.0003), daily dose ≥1000 mg (OR: 2.77, 95% CI: 1.3-6.1, P = 0.0063) and the absence of pre-existing conditions in a patient (OR: 2.55, 95% CI: 1.3-4.9, P = 0.0043) were independently associated with delayed onset. The findings were consistent when externally validated using Latin American DILI Network cases (N = 131). Likewise, drug properties of mitochondrial liability and Pauling electronegativity were associated with delayed onset, but dependent on specific host factors such as age, sex and pre-existing cardiac diseases.

Conclusions: This study demonstrated that delayed onset, a specific DILI phenotype, is explained by complex interactions among drug properties and host factors and provided mechanistic hypotheses for future studies. These findings can help improve the diagnostic capability and causality assessment.

KEY WORDS

Data mining. Hepatotoxicity, interactions, phenotype

KEY POINTS

- Symptoms of drug-induced liver injury (DILI) normally occur while the patient takes the causative drug, but can sometimes be delayed until after stopping the drug treatment (delayed onset).
- We compared drug properties and host factors between DILI cases with and without delayed onset to find factors that potentially modulate time of presentation.
- We found that patients without pre-existing diseases taking drugs with low hepatic metabolism at a daily dose ≥1000 mg are more likely to present hepatic symptoms, if developing DILI, after stopping the drug, particularly with the antibiotic amoxicillin-clavulanate.
- This information is useful when diagnosing DILI in clinical practice

INTRODUCTION

Drug-induced liver injury (DILI) is an important adverse drug reaction.[1] The exact mechanism of idiosyncratic DILI is still unknown, although it is generally accepted that DILI is a complex and multifactorial disorder. Hence, the risks and phenotypic presentations of idiosyncratic DILI are likely modulated by complex interactions among drug, host and environmental factors.[2]

Due to the lack of specific symptoms and diagnostic biomarkers, clinical diagnosis of idiosyncratic DILI largely depends on thorough clinical assessment, including exclusion of alternative causes, establishment of a compatible temporal relationship between drug administration and DILI development, and consideration of existing knowledge on clinical manifestations and frequencies of DILI caused by specific drugs. In most cases, the temporal relationship is evident when liver injury manifests during the drug treatment. However, in some cases, the clinical manifestation of liver injury, such as jaundice, is delayed and does not occur until days or even months after treatment cessation.[3] This particular phenotype is problematic as it complicates the DILI diagnosis and its clinical management. Which factors that contribute to the delayed onset phenotype is currently unknown. To explore factors associated with this particular phenotype that challenges clinical diagnosis of DILI, we designed this study using the Spanish DILI Registry database and the Latin American DILI Network. We defined DILI that manifested after the cessation of the causative drug (non-concurrent DILI development) as delayed onset and distinguished this from DILI that manifested after a long latency period (time between initiation of the causative agent and onset of liver injury) but still during the treatment (concurrent DILI development). Thus, the term "delayed" here refers to a delay in DILI manifestation relative to causative drug treatment, but not the latency time. The drug most commonly associated with delayed onset is amoxicillin-clavulanate (AC).[3] Other drugs, such as trovafloxacin, telithromycin and azithromycin, have also been associated with delayed onset.[4-6]

In this study, we aimed to identify potential delayed onset modifiers by analysing drug properties, host factors and potential interactions between these two groups using a large patient population retrieved from the Spanish DILI Registry prospective database. We also used cases from the Latin American DILI Network database as a validation cohort. Better understanding of delayed onset modifiers will aid in the identification of DILI cases with delayed onset and improve future DILI diagnosis. It may also provide insight into the specific mechanisms leading to this phenotype in idiosyncratic DILI.

MATERIALS AND METHODS

Study population

The current study population consisted of patients who met DILI criteria according to international consensus and were prospectively enrolled in the Spanish DILI Registry.[7] The operational structure of this registry has been reported elsewhere.[8] The study protocol was approved by the local ethics committee of the coordinating centre at the Hospital Universitario Virgen de la Victoria and all subjects who took part in the study gave written informed consent.

Only cases induced by a single causative drug, which was responsible for at least three cases in the Spanish DILI Registry, were included. All cases attributed to multiple drugs, herbal and dietary supplements or drugs with non-oral administration routs were excluded. Eosinophilia was defined as a serum eosinophil level >4% of white blood cells. Cases with delayed onset were defined as those that presented first symptoms (mainly dark urine, acholic stool and jaundice) at least 7 days after drug treatment cessation. Cases with first symptoms less than 7 days after drug cessation were excluded to ensure accurate classification of the study populations. Drugs were classified into two categories, with and without "delayed onset potential" (DOP and nDOP, respectively), based on known associations with delayed onset in the study cohort. Drugs ever associated with delayed onset in the Spanish DILI Registry are summarized in Table S1A along with numbers of DILI cases with and without delayed onset in the Spanish DILI registry. Drugs never associated with delayed onset are summarized in Table S1B.

Drug properties

Drug property information was retrieved from the Liver Toxicity Knowledge Base database developed and maintained at the US Food and Drug Administration's National Center for Toxicological Research.[9,10] This knowledge base accumulates comprehensive drug property information on US-marketed pharmaceuticals including various physicochemical, pharmacokinetic and toxicological properties. Information on drugs not marketed in the United States was obtained from drug summary of product characteristics at the Spanish Medicines Agency. Complementary information on specific variables such as enterohepatic circulation, and percentage of drug elimination in parent drug form was obtained from the DrugBank database.[11] Drug disposition was categorized according to biopharmaceutical drug disposition classification system (BDDCS).[12] Hepatic metabolism was classified in accordance with Lammert et al.[13] Lipoaffinity was

determined as described by Liu et al.[14] Compound electronegativity was determined using the Pauling electronegativity scale to calculate a mean electronegativity value of all atoms for each compound. High electronegativity was defined as a mean electronegativity value ≥1.016.[15] Drugs with a dose required to inhibit 50% bile salt export pump (BSEP) activity (IC50) <300 μM were considered BSEP inhibitors in the current study. Drug mitochondrial liability was considered as positive only if having positive mitochondrial toxicity findings in the literature.[16-18]

Daily dose and treatment duration were considered as host factors in this study as these parameters are dependent on patient physiological and pathological characteristics as well as the severity of the disorder.

Statistical analyses

Only drug property and host factor variables with information available in at least 70% of the drugs and cases, respectively, were included in the statistical analyses. For two-group comparisons of host factors and drug properties, we used the Student's t test or the Wilcoxon rank sum test for continuous variables, while analysis of variance with the Tukey's HSD test, or the Kruskal-Wallis test with the Mann-Whitney U test for post hoc pairwise comparisons, was used for three-group comparisons. The chi-square test was used to analyse categorical variables.

Variables associated with P-values <0.05 in the univariate analyses were considered in a multiple logistic regression model using a backward stepwise approach. The Hosmer-Lemeshow test and receiver operation characteristic curves were used to assess the predictive performance of the model. The developed model was internally validated using a bootstrapping procedure. The model was developed and assessed using the JMP statistical (JMP, SAS, Cary, NC, USA) and r (R-Studio package, version 1.01.136) software.

To explore potential drug-host interactions influencing delayed onset in DILI, a two-tier approach was applied. In the first tier analysis (screening), logistic regression models including a drug property, a host factor and their interaction term were developed using the r software. A total of 2684 models were developed to assess the combinations of 61 host factors and 44 drug properties. Drug-host pairs that showed a significant interaction with a P-value <0.05 were selected for the second tier analysis (characterization of identified drug-host interactions). Interactions with uneven parameter distributions between the two subgroups (<10% absence or presence of a specific host factor or drug

property) were not included in the second tier analysis due to concerns over unstable estimates. For each of the identified pairs, a logistic regression model was developed, having delayed onset as an outcome and the drug property as a predictor for each of the two subgroups classified by the host factor. In this second tier analysis, interactions were considered significant only when the association between delayed onset and a drug property was statistically significant in one host factor subgroup but not in the other, or when the association was statistically significant in the both subgroups but in inverse directions (preset roles). The models were developed using the R software (first tier) and the following characterization (second tier) using the JMP statistical software.

External cohort validation

Main findings from the above multivariable models were validated using an independent study cohort from the Latin American DILI Network. The same inclusion and exclusion criteria were applied to this validation cohort; only cases caused by a drug responsible for at least three cases were included and classified as delayed onset when first symptom presentation occurred at least 7 days after drug cessation. The DOP and nDOP drugs identified in the validation cohort are summarized in Table S2. The predictive performance of the delayed onset model was examined by calculating sensitivity, specificity and accuracy in the external validation cohort and comparing the values obtained with those of the original study cohort.

RESULTS

The DOP and nDOP drugs were associated with 165 and 223 DILI cases, respectively, after excluding 35 cases with first symptoms <7 days after drug cessation. Amoxicillin-clavulanate was the most prominent drug among the DOP drugs with 115 corresponding cases, of which 55 cases (48%) were associated with delayed onset. Of the 165 DILI cases caused by DOP drugs, 69 (42%) presented delayed onset of symptoms 7-52 days after treatment cessation. The remaining 96 cases presented their first symptoms while receiving the culprit drug.

Drug properties associated with DOP

Drug classes

The most frequent therapeutic class of the DOP drugs was anti-infectives (62%), especially antibacterials, followed by anti-inflammatory drugs (38%). The nDOP drugs,

on the other hand, were predominantly nervous system drugs (29%), followed by cardiovascular drugs (20%) and anti-infectives (14%).

Physicochemical properties

No significant differences were found with regard to physicochemical properties between the DOP and nDOP drugs. The DOP drugs had, however, higher molecular weight than the nDOP drugs, although the difference did not reach statistical significance (437 g/mol vs 332 g/mol) (Table 1).

Pharmacokinetic and other properties

The DOP drugs were significantly less metabolized in the liver than the nDOP drugs (28% vs 75%, P = 0.0165) and consequently had a higher proportion of drugs with ≥50% excretion in parent form (71% vs 16%, P = 0.0022). The distribution of BDDCS classes was also significantly different between the drug groups (P = 0.0080). Drugs of BDDCS class 1 (high solubility/high hepatic metabolism) and class 2 (low solubility/high hepatic metabolism) were more prevalent in the nDOP group (36% vs 0% and 41% vs 25%, respectively), while class 3 (high solubility/low hepatic metabolism) drugs predominated in the DOP group (62% vs 11%). No other properties analysed showed significant differences between the two groups (Table 1).

Host factors associated with delayed onset

Host factors were first compared between cases with and without delayed onset caused by the DOP drugs (165 cases in total) and then compared among three groups, including 223 cases caused by nDOP drugs (Table 2). The main analysis of the host factor comparisons was done using the total DOP cases including AC cases. Due to the dominance of AC among the DOP cases, we also repeated the analysis using the DOP cases without AC cases and then using only AC cases to assess the robustness of the findings. Among the cases caused by the DOP drugs, cases with delayed onset had a higher daily dose (1908 mg vs 1703 mg), more frequent jaundice (85% vs 73%), more frequent eosinophilia (35% vs 23%) and less prevalent pre-existing diseases (56% vs 67%) compared to cases without delayed onset, although the differences did not reach statistical significance. These differences were mostly found to be significant when including the cases caused by nDOP drugs. In addition, the cases induced by nDOP drugs were found to have longer duration of treatment (45 days vs 7-8 days, P = 0.0001) and

time to onset (37 days vs 7-26 days, P = 0.0001). Interestingly, an inverse correlation between daily dose and time to onset was also detected when considering the complete cohort of 388 DILI cases independent of delayed onset status, whereby higher daily dose of the causative agent required shorter time to develop DILI (Spearman þ = −0.3304).
A significant difference in type of liver injury distribution, with a notable predominance of hepatocellular injury in cases caused by nDOP drugs, was also noted when comparing the three groups (P = 0.008). However, this difference did not remain significant when AC cases were excluded from the analysis, suggesting that the difference was attributed to the high number of cholestatic/mixed cases associated with AC. Similarly, the increased frequency of jaundice in delayed onset cases was not observed when AC cases were excluded (Table S3). In addition, results using only AC cases in the comparisons are summarized in Table S4.

Multivariable model to predict delayed onset of DILI including drug properties and host factors

Drug properties and host factors that showed significant associations with delayed onset (hepatic metabolism, parent drug excretion, daily dose and pre-existing diseases) were then evaluated in a multiple logistic regression model to assess their effect sizes and predictive performance. Using a backward step elimination approach, three variables were found to increase the likelihood of delayed onset: hepatic metabolism <50% (P = 0.0003), daily culprit drug dose ≥1000 mg (P = 0.0063) and absence of pre-existing diseases (P = 0.0043) (Table 3). The calculated R2 for this model was 0.2862, and the bootstrap procedure showed minimal overfitting (0.0056). The predictive model of delayed onset resulted in 78% accuracy with 81% sensitivity and 77% specificity.

Drug-host interactions in delayed onset of DILI

Next, we sought to explore potential drug-host interactions in delayed onset of DILI. In the first exploratory analysis (screening of potential host-drug interactions), 78 of the 2684 drug-host pairs were identified as statistically significant interactions. In the subsequent characterization analysis, four pairs were further substantiated to have significant interactions in delayed onset. Three host factors, age (cut-off 60 years), sex and underlying cardiac diseases, were identified as significant effect modifiers for drugs classified by mitochondrial liability. Underlying cardiac diseases were also identified as

a significant effect modifier for drugs categorized according to Pauling electronegativity (Table 4).

External cohort validation

To externally validate the predictive model of delayed onset obtained with the Spanish study cohort, an independent cohort of 131 Latin American DILI cases were analysed. A total of two DOP drugs (three active ingredients) and 20 nDOP drugs (22 active ingredients) were identified (Table S2). The DOP and nDOP drugs were associated with 38 and 93 DILI cases respectively. Similar to the Spanish cohort, AC was the most prominent DOP drug with 29 corresponding cases, of which 22 (76%) presented delayed onset. Of the 38 cases caused by DOP drugs, 24 (63%) presented delayed onset 7-40 days after treatment discontinuation.

Comparable to the Spanish cohort, the Latin American DOP drugs were more frequently associated with low (<50%) hepatic metabolism than the nDOP drugs (100% vs 10%, P = 0.0013). Furthermore, daily dose was higher in delayed onset cases compared to non-delayed onset cases caused by either DOP drugs (1862 mg vs 1366 mg, P = 0.2137) or nDOP drugs (468 mg, P = 0.0001). The proportion of patients with pre-existing diseases was also lower in those with delayed onset; however, the difference did not reach statistical significance when compared with DILI patients without delayed onset induced by DOP (29% vs 35%), but was borderline significant when including cases caused by nDOP drugs (29% vs 55%, P = 0.0498). Applying the predictive model developed using the Spanish DILI cohort to the Latin American DILI cohort resulted in a performance of 90% accuracy with 92% sensitivity and 90% specificity.

DISCUSSION

This study focuses on identification of potential delayed onset modifiers by analysing drug properties, host factors and their interactions. Delayed onset of DILI, an intriguing presentation in idiosyncratic DILI, was defined by initial symptoms appearing first after the causative drug treatment has finished. The drugs associated with delayed onset were mainly antibiotics, with 20%-80% of the corresponding DILI cases presenting delayed onset (Table S1A). Among the DOP drugs, AC was the principal drug, corroborating earlier findings.[3,7] The fact that antibiotics appear to be more commonly associated with delayed onset is not surprising considering that antibiotics are generally prescribed for short time periods. Nevertheless, not all antibiotics produce delayed onset of DILI.

Isoniazid and ciprofloxacin, for example, did not produce this phenotype in any of the corresponding cases enrolled in the Spanish DILI registry, nor did sulfamethoxazole-trimethoprim in the Latin American validation cohort. This suggests that delayed manifestation is triggered by specific drug properties in patients with certain attributes that makes them more susceptible. Understanding the mechanisms behind this specific presentation of DILI for drugs given at short courses could improve our diagnostic capabilities and causality assessment. In this study, we have defined delayed DILI onset as the presentation of specific symptoms indicative of acute hepatic impairment, which are recognizable by patients without a physician's evaluation, at least 7 days after drug discontinuation. This definition would not be influenced by sociocultural and healthcare service factors, unlike a definition using liver enzyme elevation.

We have identified factors affecting the likelihood of delayed onset presentation in idiosyncratic DILI by analysing drug properties, host factors and their interactions using a large patient population from the Spanish DILI Registry and comprehensive drug property information from available resources in an unbiased manner. The model incorporating individual drug properties and host factors revealed three independent factors that influence the likelihood of delayed onset: hepatic metabolism (drug), daily dose (host) and pre-existing diseases (host). Less than 50% hepatic drug metabolism, a daily dose ≥1000 mg and absence of pre-existing diseases were all found to increase the likelihood of delayed onset in DILI. Contrary to previous assumptions,[19] we did not find evidence to support that this phenotype occurs due to prolonged retention of the drug or its metabolites in the body. In contrast, the DOP drugs demonstrated a tendency towards having a shorter half-life than the nDOP drugs and a similar proportion of drugs with enterohepatic circulation as the nDOP drugs. In addition, the DOP drugs were found to have significantly lower hepatic metabolism and consequently a higher proportion excreted in parent drug form. The DOP drug properties in the current study therefore speak in favour of faster excretion of the drug from the body with minimal hepatocyte interaction, rather than prolonged accumulation.

High daily dosage was identified as an important factor influencing the likelihood of delayed onset in the prediction model, with 82% of the patients presenting this phenotype having taken a daily dose of more than 1000 mg. Similar to the study conducted by the DILIN group, we found an inverse correlation between daily dose and latency period, thus the higher the daily dose the shorter the latency.[20] It is difficult to say if a higher drug dosage in fact increases the risk of delayed onset or if this is the result of the majority of

the delayed onset cases being caused by antibiotics, which are generally taken at high doses over a short time period. Another important factor found to influence the likelihood of delayed onset was pre-existing diseases, whereby the absence of pre-existing diseases increased the likelihood of this DILI manifestation. The effect of pre-existing diseases on DILI is largely unknown with limited studies performed to date. However, a recent DILI epidemiological study found that approximately 74% of patients had some form of comorbid conditions.[21] This proportion is similar to that of the current study. The negative correlation between pre-existing conditions and delayed onset could be a reason for the low proportion of DILI with this less common presentation in general, due to the common occurrence of pre-existing conditions in DILI patients. The mechanism by which pre-existing conditions could influence delayed onset is unclear. A possible explanation could be that patients without pre-existing diseases may exert sound cellular adaptation mechanisms under drug-induced cellular stress and subsequently delay the appearance of manifestations. The shorter treatment duration mostly seen in delayed onset DILI cases would collaborate with this hypothesis as the toxicological effects of the drug would eventually overtake the cellular defence mechanisms, resulting in symptomatic liver injury after drug cessation.

The three factors included in the delayed onset logistic regression model (<50% hepatic metabolism, daily culprit drug dose ≥1000 mg and absence of pre-existing diseases) were also found to be associated with delayed onset in the external Latin American validation cohort. Not surprisingly, the DOP drugs in the validation cohort were antibiotics, similar to what was found in the initial study cohort.

With regard to host factors, we did not analyse the impact of genetic variations, such as human leucocyte antigen (HLA) alleles, on delayed onset in this study. As previously shown, specific HLA alleles are associated with phenotypic differences in DILI (ie, pattern of liver injury).[22] Thus, certain genetic variants or acquired factors that regulate immune responses may contribute to delayed onset manifestation. Potential associations between delayed onset and immune-related genetic variants or immune profiles should therefore be investigated in future studies.

We also explored drug-host interactions using a two-tier approach and identified several interesting findings. These findings (Table 4) suggest that the effect of certain drug properties on delayed onset differs depending on host factors. That is, drug properties and host factors on their own may not have the same effect on delayed onset manifestation as when combined. For instance, drugs without potential mitochondrial liability decrease

likelihood of delayed onset manifestation in patients with an age of <60 years but not in patients with an age of ≥60 years. A similar relationship was apparent for sex and mitochondrial liability, whereby the lack of potential mitochondrial liability in the causative drug decreased the likelihood of delayed onset manifestation in women, but not in men. In patients with cardiac comorbidities, drugs without mitochondrial liability and drugs with high Pauling electronegativity increase the likelihood of delayed onset manifestation, while these drugs have opposite effects in patients without cardiac comorbidities. Mechanisms explaining these interactions are unknown. Further studies are warranted to investigate specific interplay between host attributes and specific drug properties in delayed DILI manifestation.

Our study has strengths. It is the first study to investigate host factors, drug properties and their interactions influencing a DILI phenotype by using a large DILI data set from the Spanish DILI Registry. Further, the study findings were externally validated using an independent data set from the Latin American DILI Network. The well-characterized DILI cases assessed in this study and the strict definition used for delayed onset allowed the identification of significant factors associated with delayed manifestation of DILI and generated hypotheses pertaining to delayed onset in DILI manifestation. Our study also has weaknesses. No blood analyses were performed on the delayed onset patients at the time of the last treatment dose. Hence, while we assume that the liver profile was normal at this time point, it cannot be validated. The size of the delayed onset population was not sufficient to assess impacts of specific co-medications or their interactions. In addition, our current methodology does not allow assessing multifactorial risks. Further developments of novel investigational approaches are needed to determine how host factors, drug properties and their specific interactions play a role in diverse DILI manifestations. Meanwhile, this study provides a set of characteristics linked to delayed onset, including information on drug classes with DOP, drug exposure time, time to onset and time from drug discontinuation to symptom onset and host factor modulators. Altogether, these features can point towards certain drugs as causal agents in the adjudication process even when a patient is no longer taking the drug and improve our DILI diagnosis in future.

REFERENCES

1. Kullak-Ublick GA, Andrade RJ, Merz M, et al. Drug-induced liver injury: recent advances in diagnosis and risk assessment. Gut. 2017;66:1154-1164.

2. Chen M, Suzuki A, Borlak J, Andrade RJ, Lucena MI. Drug-induced liver injury: interactions between drug properties and host factors. J Hepatol. 2015;63:503-514.

3. Lucena MI, Andrade RJ, Fernández MC, et al. Determinants of the clinical expression of amoxicillin-clavulanate hepatotoxicity: a prospective series from Spain. Hepatology. 2006;44:850-856.

4. Lucena MI, Andrade RJ, Rodrigo L, et al. Trovafloxacin-induced acute hepatitis. Clin Infect Dis. 2000;30:400-401.

5. Brinker AD, Wassel RT, Lyndly J, et al. Telithromycin-associated hepatotoxicity: clinical spectrum and causality assessment of 42 cases. Hepatology. 2009;49:250-257.

6. Martinez MA, Vuppalanchi R, Fontana RJ, et al. Clinical and histologic features of azithromycin-induced liver injury. Clin Gastroenterol Hepatol. 2015;13:369-376.

7. Aithal GP, Watkins PB, Andrade RJ, et al. Case definition and phenotype standardization in drug-induced liver injury. Clin Pharmacol Ther. 2011;89:806-815.

8. Andrade RJ, Lucena MI, Fernández MC, et al. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. Gastroenterology 2005;129:512-521.

9. Chen M, Zhang J, Wang Y, et al. The liver toxicity knowledge base: a systems approach to a complex end point. Clin Pharmacol Ther. 2013;93:409-412.

10. Hong H, Chen M, Ng HW, Tong W. QSAR models at the US FDA/NCTR. Methods Mol Biol. 2016;1425:431-459.

11. Wishart DS, Knox C, Guo AC, et al. DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic Acids Res 2006;34:D668-D672.

12. Benet LZ, Broccatelli F, Oprea TI. BDDCS applied to over 900 drugs. AAPS J. 2011;13:519-547.

13. Lammert C, Bjornsson E, Niklasson A, Chalasani N. Oral medications with significant hepatic metabolism at higher risk for hepatic adverse events. Hepatology. 2010;51:615-620.

14. Liu R, Sun H, So SS. Development of quantitative structure-property relationship models for early ADME evaluation in drug discovery. 2. Blood-brain barrier penetration. J Chem Inf Comput Sci. 2001;41:1623-1632.

15. Matsunaga N, Rogers DW, Zavitsas AA. Pauling's electronegativity equation and a new corollary accurately predict bond dissociation enthalpies and enhance current understanding of the nature of the chemical bond. J Org Chem. 2003;68:3158-3172.
16. Persson M, Løye AF, Mow T, Hornberg JJ. A high content screening assay to predict human drug-induced liver injury during drug discovery. J Pharmacol Toxicol Methods. 2013;68:302-313.
17. Porceddu M, Buron N, Roussel C, Labbe G, Fromently B, Borgne- Sanchez A. Prediction of liver injury induced by chemicals in human with a multiparametric assay on isolated mouse liver mitochondria. Toxicol Sci. 2012;129:332-345.
18. O'Brien PJ, Irwin W, Diaz D, et al. High concordance of drug-induced human hepatotoxicity with in vitro cytotoxicity measured in a novel cell-based model using high content screening. Arch Toxicol. 2006;80:580-604.
19. Kaplowitz N. Drug-induced liver injury. Clin Infect Dis. 2004;38(Suppl 2):S44-S48.
20. Vuppalanchi R, Gotur R, Reddy KR, et al. Relationship between characteristics of medications and drug-induced liver disease phenotype and outcome. Clin Gastroenterol Hepatol. 2014;12:1550-1555.
21. Baekdal M, Ytting H, Skalshøi KM. Drug-induced liver injury: a cohort study on patients referred to the Danish transplant center over a five year period. Scand J Gastroenterol. 2017;52:450-454.
22.Stephens C, López-Nevot MÁ, Ruiz-Cabello F, et al. HLA alleles influence the clinical signature of amoxicillin-clavulanate hepatotoxicity. PLoS ONE. 2013;8:e68111.

Table 1. Physicochemical, pharmacokinetic and pharmacodynamic properties of drugs with (DOP) and without (nDOP) delayed onset potential

	DOP active ingredients N=9*	**nDOP active ingredients N=36***	***p***
Physicochemical			
Molecular weight, mean g/mol ±SD	437 ±197	332 ±130	0.0704
Total number of rings, mean ±SD	2.7 ±1.56	2.5 ±1.23	0.6483
Number of aromatic rings, mean ±SD	0.9 ±0.6	1.0 ±0.1	0.6337
Number of heterorings, mean ±SD	1.9 ±1.4	1.3 ±0.9	0.1606
Length of chain, mean atoms ±SD	3.1 ±1.8	5.7 ±5.3	0.2198
Length of aliphatic chain, mean ±SD	0.6 ±1.8	2.2 ±3.0	0.1603
High Pauling electronegativity, n (%)	3 (37)	4 (11)	0.0649
Pharmacokinetics			
Half-life, median hours (IQR)	3.2 (1.3-31)	6 (2.9-17)	0.8427
Lipophilia, median LogP (IQR)	1.1 (-1.3-2.1)	2.7 (0.8-3.8)	0.0847
Proportion of plasma protein binding , median (IQR)	63 (28-95)	90 (43-96)	0.5620
Hepatic metabolism ≥50%, n (%)	2 (28)	27 (75)	**0.0165**
Parent drug excretion ≥50%, n (%)	5 (71)	5 (16)	**0.0022**
Enterohepatic circulation, n (%)	3 (33)	13 (39)	0.7400
Reactive metabolite formation, n (%)	5 (55)	20 (57)	0.9317
Mitochondrial liability, n (%)	4 (50)	22 (63)	0.5022
BDDCS, n (%)			
class 1 (↑solub / ↑hep met)	-	13 (36)	
class 2 (↓solub / ↑hep met)	2 (25)	15 (41)	**0.0080**
class 3 (↑solub / ↓hep met)	5 (62)	4 (11)	
class 4 (↓solub / ↓hep met)	1 (13)	4 (11)	
Pharmacodynamics			
BSEP inhibitor, n (%)	1 (25)	13 (56)	0.2442

All percentages have been calculated based on the number of drugs with available information (>70%).

BDDCS, Biopharmaceutical drug disposition classification system; solub, solubility; hep met, hepatic metabolism; ↑, high; ↓, low; BSEP, bile salt export pump.

*45 active ingredients corresponding to 43 drug treatments.

Table 2. Demographics and clinical characteristics of drug-induced liver injury (DILI) cases with and without delayed onset (DO), induced by drugs with delayed onset potential (DOP) and drugs without DOP (nDOP)

	DOP drugs			nDOP drugs	
	DO (69)	**No DO (96)**	p^*	**No DO (223)**	$\boldsymbol{p^{**}}$
Demographics					
Age, median years (IQR)	62 (46-70)	61 (46-71)	0.8087	60 (43-70)	0.6294
Women, n (%)	31 (45)	50 (52)	0.3644	109 (49)	0.6622
Clinical presentation					
Duration of treatment, median days (IQR)	7[#] (6-11)	8[#] (6-21)	0.1289	45 (23-103)	**0.0001**
Time to onset, median days (IQR)	26[#] (18-34)	7[#] (4-13)	**0.0001**	37 (18-90)	**0.0001**
Daily dose, mean mg ±SD	1908 ±962[#]	1703 ±1037[#]	0.2105	379 ±597	**0.0001**
Jaundice, n (%)	58 (85)[#]	70 (73)	0.0592	147 (66)	**0.0104**
Eosinophilia, n (%)	24 (35)	22 (23)	0.0822	45 (21)	0.0665
Pre-existing diseases, n (%)	39 (56)[#]	64 (67)[#]	0.1844	192 (86)	**0.0001**
Hepatic diseases	2 (2.9)	4 (4.2)	0.6678	13 (5.8)	0.5707
Biochemical values, mean xULN (range)					
TBL	8.5 (0.4-33)	6.5 (0.3-28)	0.0615	7.2 (0.1-46)	0.2249
AST	10 (1.0-97)[#]	12 (0.6-103)[#]	0.5293	17 (0.2-113)	**0.0144**
ALT	15 (0.8-115)	15 (0.8-98)[#]	0.8285	11 (1.0-123)	**0.0305**
ALP	2.6 (0.3-15)	2.4 (0.4-19)	0.6004	2.3 (0.3-22)	0.6937
Pattern of liver injury, n (%)			0.9742		**0.0008**
Hepatocellular	32 (46)	41 (45)		149 (67)	
Cholestatic	16 (23)	22 (24)		36 (16)	
Mixed	21 (30)	29 (31)		37 (16)	
Severity, n (%)			0.4488		**0.0327**
Mild	11 (16)	22 (24)		71 (33)	
Moderate	52 (75)	63 (68)		118 (55)	
Severe	4 (5.8)	3 (3.2)		18 (8.3)	
Fatal/Transplant	2 (2.9)	5 (5.4)		9 (4.2)	

All percentages have been calculated based on the number of cases with available information (>70%).
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBL, total bilirubin; xULN, times upper limit of normal.

*comparison between two groups: DILI cases induced by DOP drugs (with and without DO)
**comparison between three groups: DILI cases induced by DOP drugs (DO and no DO) and nDOP drugs
[#]$p<0.05$ compared to cases without delayed onset caused by nDOP drugs (post-hoc pairwise comparisons).

Table 3. Predictive model for delayed onset in drug-induced liver injury based on independent drug properties and host factors

	OR	***p***	**95% CI**
Hepatic metabolism <50%	11.06	0.0003	4.38-32.20
Daily culprit drug dose ≥1000 mg	2.77	0.0063	1.33-6.06
Absence of pre-existing diseases	2.55	0.0043	1.34-4.86

Table 4. Host factors modifying effects of drug properties on the likelihood of manifesting delayed onset in drug-induced liver injury

		Total cases, N	Delayed onset, N	%	OR [95% CI], p-value
Age ≥60 years					
Potential mitochondrial liability	Yes	168	34	20	-
	No	30	6	20	0.98 [0.34-2.47], 0.9761
Age <60 years					
Potential mitochondrial liability	Yes	133	27	20	-
	No	35	1	3	0.12 [0.06-0.57], 0.0044
Male					
Potential mitochondrial liability	Yes	160	32	20	-
	No	28	6	21	1.09 [0.37-2.77], 0.8630
Female					
Potential mitochondrial liability	Yes	141	29	21	-
	No	37	1	3	0.11 [0.01-0.53], 0.0027
Absence of underlying cardiac diseases					
Potential mitochondrial liability	Yes	266	60	22	-
	No	57	4	7	0.26 [0.08-0.66], 0.0033
Presence of underlying cardiac diseases					
Potential mitochondrial liability	Yes	35	1	3	-
	No	8	3	37	20.40 [2.15-461.5], 0.0084
Absence of underlying cardiac diseases					
Pauling electronegativity	Low	295	60	20	-
	High	48	5	10	0.46 [0.15-1.10], 0.0838
Presence of underlying cardiac diseases					
Pauling electronegativity	Low	40	2	7	-
	High	5	2	40	12.67 [1.19-145], 0.0363

Supplementary table 1

A) Eight drugs (9 active ingredients) associated with delayed onset of drug-induced liver injury in the Spanish DILI Registry

Drug	N	Delayed onset	No delayed onset	BDDCS	≥50% hepatic metabolism	≥50% of drug excreted in parental form
Amoxicillin-clavulanate	115	55	60	3/3	No/-	Yes/-
Azithromycin	3	1	2	3	No	Yes
Droxicam*	4	1	3	-	-	-
Erythromycin	5	1	4	3	No	Yes
Ibuprofen	16	3	13	2	Yes	No
Levofloxacin	8	2	6	3	No	Yes
Nimesulide*	9	2	7	2	Yes	No
Trovafloxacin	5	4	1	4	No	Yes

Drugs that were considered as culprits in three or more cases in the registry and were associated with delayed onset in at least one case are listed above. A total of 165 cases, including 69 cases with delayed onset and 96 cases without delayed onset, were related to the 8 listed drugs.
* Droxicam, a prodrug of piroxicam, and nimesulide are currently withdrawn from the Spanish market due to DILI.
BDDCS: Biopharmaceutical drug disposition classification system

B) Thirty-five drugs (36 active ingredients) that were never associated with delayed onset of drug-induced liver injury in the Spanish DILI Registry

Drug	N	Drug	N
Acarbose	3	Flutamide	20
Allopurinol	3	Fluvastatin	11
Amineptine*	3	Isoniazid	20
Amiodarone	3	Lovastatin	3
Atorvastatin	12	Methotrexate	6
Azathioprine	9	Moxifloxacin	3
Bentazepam	7	Naproxen	3
Captopril	6	Olanzapine	3
Carbamazepine	7	Omeprazole	4
Chlorpromazine	3	Orlistat	3
Ciprofloxacin	5	Paroxetine	9
Clomethiazole	4	Phenytoin	3
Clopidogrel	4	Sulfamethoxazole/trimethoprim	4
Cloxacillin	3	Sulfasalazine	3
Disulfiram	5	Thiamazole	7
Ebrotidine*	18	Ticlopidine	12
Enalapril	3	Valproic acid	7
Fenofibrate	4		

Drugs that were considered as culprits in three or more cases in the registry and were never associated with delayed onset are listed above. A total of 223 cases were related to the 35 listed drugs.
*Currently withdrawn from the Spanish market due to DILI

Supplementary table 2

A) Two drugs (3 active ingredients) associated with delayed onset of drug-induced liver injury in the Latin American DILI registry

Drug	N	Delayed onset	No delayed onset	BDDCS	≥50% hepatic metabolism	≥50% of drug excreted in parental form
Amoxicillin-clavulanate	29	22	7	3/3	No/-	Yes/-
Nitrofurantoin	9	2	7	4	No	Yes

Drugs that were considered as culprits in three or more cases in the Latin American registries and were associated with delayed onset in at least one case in the Latin American registry are listed above. A total of 38 cases, including 24 cases with delayed onset and 14 cases without delayed onset, were related to the two listed drugs.
BDDCS: Biopharmaceutical drug disposition classification system

B) Twenty drugs (22 active ingredients) that were never associated with delayed onset of drug-induced liver injury in the Latin American DILI registry

Drug	N	Drug	N
Albendazole	3	Isoniazid	4
Atorvastatin	4	Methyldopa	5
Azathioprine	4	Metronidazole	3
Carbamazepine	7	Nimesulide	7
Cyproterone	8	Phenytoin	3
Diclofenac	10	Propafenone	3
Drospirenone and estrogen	3	Propylthiouracil	3
Fenofibrate	4	Sulfamethoxazole/trimethoprim	3
Flutamide	4	Thiamazole	4
Ibuprofen	7	Valproic acid	4

Drugs that were considered as culprits in three or more cases in the Latin American registries and were never associated with delayed onset are listed above. A total of 93 cases were related to the 20 listed drugs.

Supplementary table 3. Demographics and clinical characteristics of DILI cases with and without delayed onset (DO), induced by drugs with delayed onset potential (DOP) and drugs without delayed onset potential (nPDO), excluding all cases caused by amoxicillin-clavulanate

	DOP drugs			nDOP drugs	
	DO (14)	**No DO (36)**	***p****	**No DO (223)**	***p*** **
Demographics					
Age, median years (IQR)	56 (34-69)	54 (43-64)	0.7786	60 (43-70)	0.8716
Women, n (%)	6 (43)	23 (64)	0.1767	109 (49)	0.2077
Clinical presentation					
Duration of treatment, median days (IQR)	8 (5-15)	15 (6-35)	0.0744	45 (23-103)	**0.0001**
Time to onset, median days (IQR)	26 (19-30)	10 (4-29)	**0.0114**	37 (18-90)	**0.0001**
Daily dose, mean mg ±SD	534±481	700 ±567	0.3405	379 ±597	**0.0108**
Jaundice, n (%)	11 (79)	21 (58)	0.1807	147 (66)	0.3760
Eosinophilia, n (%)	6 (43)	6 (17)	0.0515	45 (21)	0.1242
Positive autoantibodies titers, n (%)	-	7 (19)	0.0752	48 (27)	0.0588
Pre-existing diseases, n (%)	7 (50)	21 (58)	0.5940	192 (86)	**0.0001**
Hepatic diseases	1 (7)	1 (3)	0.4794	13 (6)	0.7286
Pattern of liver injury, n (%)			0.2470		0.3989
Hepatocellular	11 (79)	18 (53)		149 (67)	
Cholestatic	1 (7)	7 (21)		36 (16)	
Mixed	2 (14)	9 (26)		37 (16)	
Severity, n (%)			0.4881		0.2337
Mild	4 (29)	12 (34)		71 (33)	
Moderate	9 (64)	19 (54)		118 (54)	
Severe	1 (7)	-		18 (8)	
Fatal/Transplant	-	4 (11)		9 (4)	

*comparison between two groups: DILI cases induced by DOP drugs (with and without DO)
**comparison between three groups: DILI cases induced by DOP drugs (DO and no DO) and nDOP drugs
p<0.05 compared to cases without delayed onset caused by nPDO drugs (post-hoc pairwise comparisons)
All percentages have been calculated based on the number of cases with available information (>70%).

Supplementary table 4. Demographics and clinical characteristics of amoxicillin-clavulanate DILI cases with and without delayed onset (DO) and drugs without DO potential (nDOP)

	Amoxicillin-clavulanate			nDOP drugs	
	DO (55)	**No DO (60)**	***p****	**No DO (223)**	***p*** **
Demographics					
Age, median years (IQR)	62 (47-70)	65 (53-75)	0.4778	60 (43-70)	0.2651
Women, n (%)	25 (45)	27 (45)	0.9610	109 (49)	0.8146
Clinical presentation					
Duration of treatment, median days (IQR)	8[#] (6-12)	8[#] (6-13)	0.8661	45 (23-103)	**0.0001**
Time to onset, median days (IQR)	26[#] (18-34)	6[#] (4-8)	**0.0001**	37 (18-90)	**0.0001**
Daily dose, mean mg ±SD	2277 ±681[#]	2292 ±760[#]	0.9188	379 ±597	**0.0001**
Jaundice, n (%)	47 (87)[#]	49 (82)	0.4324	147 (66)	**0.0022**
Eosinophilia, n (%)	18 (33)	16 (27)	0.4372	45 (21)	0.1800
Positive autoantibodies titers, n (%)	7 (13)	12 (20)	0.2942	48 (27)	0.0750
Pre-existing diseases, n (%)	32 (58)[#]	43 (72)[#]	0.1293	192 (86)	**0.0001**
Hepatic diseases	1 (2)	3 (5)	0.3523	13 (6)	0.4756
Biochemical values, mean xULN (range)					
TBL	8.6 (0.37-32)	6.4 (0.48-21)	0.0419	7.2 (0.14-46)	0.2423
AST	8.8 (1.0-97) [#]	10 (0.60-103) [#]	0.6782	17 (0.2-113)	**0.0021**
ALT	12 (0.84-115)[#]	14 (0.85-69)[#]	0.6865	11 (0.98-123)	**0.0058**
ALP	2.6 (0.47-15)	2.4 (0.43-19)	0.6325	2.3 (0.33-22)	0.6410
Pattern of liver injury, n (%)			0.9871		**0.0001**
Hepatocellular	21 (38)	23 (40)		149 (67)	
Cholestatic	15 (27)	15 (26)		36 (16)	
Mixed	19 (35)	20 (34)		37 (16)	
Severity, n (%)			0.8506		**0.0105**
Mild	7 (13)	10 (17)		71 (33)	
Moderate	43 (78)	44 (76)		118 (54)	
Severe	3 (5)	3 (5)		18 (8)	
Fatal/Transplant	2 (4)	1 (2)		9 (4)	

*comparison between two groups: DILI cases induced by DOP drugs (with and without DO)
**comparison between three groups: DILI cases induced by DOP drugs (DO and no DO) and nDOP drugs
[#]$p<0.05$ compared to cases without delayed onset caused by nDOP drugs (post-hoc pairwise comparisons)