Control compounds used to validate in vitro models of idiosyncratic drug-induced liver injury: a systematic review

Segovia-Zafra A, Villanueva-Paz M, Serras A, Matilla-Cabello G, Bodoque A, Di Zeo-Sánchez D, Niu H, Álvarez-Álvarez I, Godek S, Milisav I, Fernández-Checa JC*, Lucena MI*, Cubero FJ, Miranda J, Nelson L, Andrade RJ*.

Background and Aims: Idiosyncratic Drug-Induced Liver Injury (DILI) encompasses the unpredictable damage that drugs, herbs, and dietary supplements may cause to the liver. When studying the prediction of DILI at preclinical stages, the choice of a validated system is of crucial importance. The present study seeks to provide a list of both DILI positive and negative control compounds. This list arises from a systematic analysis of the existing literature, supported by clinical evidence collected in both national and international DILI registries and endorsed by a committee of experts from the ProEuroDILI Network (COST Action 17112).

Method: This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. Eligible literature published to June 1st, 2022 was identified through a search in PubMed, Embase, Web of Science, and Scopus. Only peer-reviewed original articles focused on studying the onset of DILI by using preclinical *in vitro* human models were included. The reliability of the studies was assessed using a modified version of the software-based "Toxicological data Reliability Assessment Tool" (ToxRTool). Drugs most commonly used as DILI-positive and negative controls in the literature were selected for in-depth analysis.

Results: The search strategy retrieved a total of 2936 studies from the above-mentioned databases. After screening, 2885 studies were excluded, as they were duplicates or did not meet the inclusion criteria. 51 articles were included in the study. Most studies were categorized as reliable without restrictions (58.6%). The mean number of drugs tested was 55 DILI-positive and 30 DILI-negative compounds. Diclofenac was the drug most drug used as DILI-positive control (88%), followed by troglitazone (80%) and flutamide (71%). Regarding DILI-negative controls, buspirone (49%), dexamethasone (41%), and diphenhydramine (35%) were the most tested compounds. Acetylsalicylic acid, fluoxetine, or warfarin were widely used as DILI-negative controls (33%, 25%, and 22%, respectively), but also as DILI-positive compounds (22%, 20%, and 16%). Moreover, up to 19% of the drugs used as DILI-negative controls had clinical hepatotoxicity cases reported within different DILI registries. The drug concentrations used varied remarkably. Although 49% of studies chose the drug concentrations based on the C_{max} values, the C_{max} assumed for the same drug in different studies diverged. The majority of studies assessed the effect of drugs in the short term (≤ 72 h; 71%). Cytotoxicity was the endpoint most evaluated (82%). Nevertheless, several studies included the assessment of functional parameters, such as biotransformation activity (20%), albumin (20%), or urea secretion (12%). A few studies included mechanistic endpoints such as cholestasis (24%) or mitochondrial damage (22%).

Conclusion: This systematic study has shown a lack of consensus in terms of in vitro DILI modelling. Since no single system serves as a universal test for the multifactorial process of DILI, a portfolio of robust and well-characterized predictive DILI platforms with a well-defined purpose is required. Moreover, there is a need for consensus about the reference drugs to be used for the DILI assay validations, including recommendations about the range of concentrations to test as well as criteria for interpreting the data.

Funding: PI19/00883, PEMP-2020-0127, PI21/01248, P18-RT-3364, PI-0310-2018, ISCIII CIBERehd, POSTDOC_21_00780, CD20/00083, CD2100198.

Figure:

	COMPOUND	N° articles using it as DILI positive (%)	N° articles using it as DILI negative (%)
COMPOUNDS MOST USED AS DILI POSITIVE CONTROLS	Diclofenac	45 (88.2%)	0 (0%)
	Troglitazone	41 (80.4%)	0 (0%)
	Flutamide	36 (70.6%)	0 (0%)
	Amiodarone	35 (68.6%)	0 (0%)
	Acetaminophen (APAP)/ Paracetamol	33 (64.7%)	0 (0%)
	Ketoconazole	33 (64.7%)	0 (0%)
	Nefazodone	29 (56.9%)	0 (0%)
	Tamoxifen	29 (56.9%)	0 (0%)
	Chlorpromazine	27 (52.9%)	1 (1.9%)
	Isoniazid	27 (52.9%)	1 (1.9%)
COMPOUNDS MOST USED AS DILI NEGATIVE CONTROLS	Buspirone	3 (5.9%)	25 (49%)
	Dexamethasone	0 (0%)	21 (41.2%)
	Diphenhydramine	0 (0%)	18 (35.3%)
	Acetylsalicylic acid/Aspirin	11 (21.6%)	17 (33.3%)
	Isoproterenol	0 (0%)	17 (33.3%)
	Caffeine	0 (0%)	15 (29.4%)
	Propranolol	5 (9.8%)	15 (29.4%)
	Flumazenil	0 (0%)	14 (27.5%)
	Primidone	0 (0%)	14 (27.5%)
	Streptomycin	0 (0%)	14 (27.5%)