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Building a Spanish-Latin American network on drug induced liver injury: much to get from a joint collaborative initiative

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INTRODUCTION

Latin America lacks a centralized database registry capable of reliably accounting for the incidence and particular characteristics of drug-induced liver damage within the region. Collaborative efforts, consensus-based diagnostic criteria and a standardized nomenclature are the essential components to perform this task.

The present work focuses on the steps taken by Latin American countries to tackle their present isolation and set up a collaborative network together with Spain in order to further understand and investigate the complex underlying mechanism of drug-induced liver injury (DILI).

DIFFICULTIES IN CLINICAL RESEARCH IN THE FIELD OF DRUG INDUCED LIVER INJURY

DILI is a challenging entity responsible for multiple clinical manifestations and an even larger spectrum of clinical severity. For most pharmacologic agents, hepatotoxicity is extremely rare and has been estimated to occur in 1 in 10,000 to 1 in 100,000 of the individuals exposed to the drug, and for a large number of compounds the risk is probably even lower. Recent data from US showed that antibiotics were the drug class most frequently associated with non-fulminant drug-induced hepatitis[1] (amoxicillin/clavulanic acid, minocycline, nitrofurantoin, trimethiprim-sulfamethoxazole, telithromycin and trovafloxacin). Antimicrobials were also the most common cause of DILI observed in a Spanish Registry[2] and in United Kingdom and French studies.[3,4]

The diagnosis of DILI requires physicians' awareness of the condition to generate *suspicion*, along with clinical skills to critically evaluate the heterogeneous phenotypes of liver toxicity. Indeed, establishing *causality* between drugs and liver damage is a difficult task, particularly in the frequent scenario of patients receiving multiple medications including more than one potential hepatotoxic drug. Likewise, the presence of drug or alcohol abuse as well as that of underlying liver diseases might turn the process of establishing causality extremely challenging, not to mention the elevated risk of generating inaccurate assessments if stringent criteria are not applied when diagnosing DILI. In addition, DILI *lacks specific markers*, and intentional drug

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re-challenge that could confirm a hepatotoxicity suspicion of is unethical and therefore not recommendable. The aforementioned confounding factors in conjunction with the continuously *increasing number of reports implicating pharmacologic agents and herbs* in liver toxicity make the field of DILI particularly intriguing and difficult. The main role of causality scale evaluations is to assess the temporal relationship between drug initiation time and development of abnormal liver enzymes and to exclude other causes of liver injury. Histology is usually not helpful as it determines the degree and extent of liver damage rather than the etiology.

Moreover, several circumstances such as pharmaceutical policies and prescription habits, ethnicity, environmental and genetic factors appear to modulate not only the incidence, but also the clinical form and DILI severity. A clear example of these differences is the high frequency of liver damage induced by ibuprofen found by the Spanish Registry of Hepatotoxicity[2] compared with the low incidences reported in studies outside Spain.[5] A similar situation was documented concerning nimesulide-induced liver-damage in Argentina, Ireland, Finland, Spain and Uruguay over the last 20 years, where a high frequency of cholestatic hepatitis and severe liver impairment was described, which notably differs from that reported in several other European countries.[6-9] Unfortunately, due to the absence of controlled trials and incomplete post-marketing studies the true incidence of hepatotoxicity is often underestimated. Systematic reporting of DILI instances to regulatory authorities (the so-called yellow card) has failed in providing true incidence estimates mainly because of underreporting. Furthermore, reporting physicians tend to focus on instances associated with the use of new drugs and on the more severe reactions, ignoring the fact that an important number of compounds induce milder phenotypes including asymptomatic hypertransaminasemia. This prompted a recent expert consensus meeting to establish a threshold for fulfilling liver damage criteria according to alanine transaminase (ALT) levels in folds of upper limit of normal (ULN) values.[10] Besides this, systematic reporting is inaccurate and roughly 50% of suspected incidences may later on be excluded after appropriate ascertainments of alternative causes.[11]

Diclofenac-induced hepatotoxicity is one of the most conclusive examples of how the medical knowledge of drug toxicity can be improved in the post authorization phase when prospective controlled trials have ended and the drug gets into the market. Diclofenac had historically been linked to a low degree of liver involvement. However, in 1995 Banks and co-workers reported an analysis of 180 cases submitted to the FDA between 1988 and 1991; and proposed for the first time that the risk of diclofenac-induced liver injury was significantly higher than previously suspected.[12] This concept was recently addressed by Laine, *et al.*, who conducted the largest liver related diclofenac study ever reported, as part of the Multinational Etoricoxib and Diclofenac Arthritis Long-Term [MEDAL] program.[13] They prospectively analyzed diclofenac-induced liver reactions in 17,289 patients diagnosed of osteoarthritis or rheumatoid arthritis and over 50 years. They were randomized to receive either diclofenac (150 mg daily) or etoricoxib (60 or 90 mg daily). Values of ALT > 3 xULN were observed in over 3% of arthritic patients with a regular drug intake. It is interesting to point out that ALT values higher than 10 xULN were only identified in 0.5% of the cases. The incidence of diclofenac liver-related hospitalizations in this study was 0.023% accounting for 16 per 10^5 patient-year.

Conversely, poorly designed post-marketing studies can generate unreliable data on drug induced liver toxicity. The flaw in these analyses often resides in the miscalculation of the number of exposed individuals whenever a drug is commercially available.

Nevertheless, for many drugs no post marketing studies have yet been performed. The only way to overcome this lack of information is by stimulating the creation of DILI Registries. That was the rationale that encouraged several of us to foster a mutual and coordinated database system on our continent, the so called Latin American Registry of hepatotoxicity.

WHY IS THERE A NEED TO SET UP A SPANISH-LATIN AMERICAN DILI NETWORK?

The lack of a Latin American registry where physicians can centralize reports on hepatotoxic events occurring in the region constitutes a major epidemiological deficiency in the liver toxicity field within our continent. Information on hepatotoxicity in Latin American is scanty and usually comes from case reports or small series of patients. The lack of information regarding the incidence and particular characteristics of DILI makes it necessary to implement a *data registry system* that would allow us to include all DILI data recorded prospectively over time. Physicians using a structured and uniform report

form protocol and methodologically trained would provide valuable information to identify the characteristics of patients, drugs or herbals more frequently involved in DILI, pattern of liver injury and outcome. Furthermore, Latin America represents a group of 23 countries composed by different ethnicities (Hispanic, Black, Mestizo, Amerindian and European). Such an ethnical diversity within the same geographical area is both a challenge and a chance to enhance the knowledge on the ethnic influence in genetic susceptibility as well as phenotypes of drug induced liver damage.[14]

It is very important to highlight that this Spanish-Latin American project will be supported by the University of Malaga and the Spanish DILI Registry, which over the past years has exhibited extensive experience in hepatic adverse events induced by drugs. Another widely recognized international hepatotoxicity registry is the DILIN (Drug Induced Liver Injury Network) in the United States active since 2003[15] which, along the Spanish registry, are the only ones collecting cases on a prospective basis. Collaborative groups should not only contribute to improve the medical knowledge on liver toxicity but also intend to fulfill other specific issues such as:

- Conduct drug surveillance studies.
- Perform phenotypic and genotypic studies in order to identify and individualize pathogenic mechanisms specifically related to drug susceptibility, involved in different populations and ethnicities.
- Establish new consensus and to discuss new definitions of DILI phenotypes and outcomes.
- To further refine hepatotoxicity causality criteria by better weighing clinical information available. Adequate management of these factors will have a significant impact on clinical practice and would have an important bearing on Public Health.

HOW SHOULD THE SPANISH-LATIN AMERICAN NETWORK OPERATE?

To date several Latin American countries (Argentina, Uruguay, Brazil, Peru, Mexico, Chile and Venezuela) have already joined this project and have shown genuine commitments to collaboratively build a Latin American network of Hepatotoxicity. In addition to the University of Malaga, ALEH (Latin American Association of Hepatology) has supported this project allowing us to open a hepatotoxicity section on its website.[16] This link will have a specific window in which physicians will be able to upload both clinical and laboratory information related to potential liver toxicity cases and that could feed a data base.

One of the most ambitious objectives of this project is to search for genetic susceptibility biomarkers. To attain this goal, biological samples are being collected to build a biobank for mechanistic studies. Samples will then be analyzed by trained staff to identify links between hepatotoxicity, genetic markers and relevance within different ethnic groups. In order to retrieve sufficient number of patients with hepatotoxicity analyses will be done together with Spanish samples and also in concerted International collaborations, such as the International Drug-Induced Liver Injury Consortium (IDILIC).[17]

The encouraging results in the literature on genetic studies in DILI patients highlight the different behavior of a given drug according to the genetic host. Furthermore, a same drug can express different types and severity of liver damage according to ethnicity.[18]

Using a candidate gene approach the more consistent association in DILI has been observed for N-acetyltransferase 2 (NAT2). The NAT2 association relates to liver injury induced by isoniazid, with most published studies finding an increased risk of injury in slow acetylators lacking NAT2 enzyme activity, presumably because of the accumulation of toxic metabolites.[19]

On the other hand, human leukocyte antigen (HLA) class I and II genes have also been extensively investigated in DILI patients. Associations between amoxicillin-clavulanate-related liver injury and the HLA class II DRB1*1501-DQB1*0602 haplotype and flucloxacillin-related injury and the HLA class I B*5701 allele were recently published.[20,21] A genome wide analysis and high-resolution genotyping of the HLA region performed in a larger cohort with more diverse amoxicillin-clavulanate DILI patients (American, north European and south European populations), identified that both class I and II HLA alleles influence amoxicillin-clavulanate susceptibility for developing DILI. The HLA-DRB1*1501-DQB1*0602 association confirmed earlier findings, but the novel class I association points to an independent role for HLA-A*0201 and possibly also for HLA-B*1801 exclusively in the Spanish population.[22,23] These results affirm the importance of the adaptive immune response in drug-induced idiosyncratic liver injury and reinforce the important ethnic influence on genetic susceptibility. The associations found within HLA class I and II genes seem to be drug specific,

though some apparently functionally and structurally unrelated compounds show genetic associations with the same alleles. The underlying mechanism for HLA associations is likely to involve T-cell responses to either drug-protein adducts or to the drug itself, but needs further investigation.

Although data are still limited, recent candidate gene studies indicate that genes from several of the metabolic, transporter, detoxifying and mitochondrial injury in the up-stream pathways also contribute to DILI susceptibility. The majority of these studies involve small number of cases, but a few of the associations reported have been independently replicated.[24] Larger series of cases are currently being collected and, with the more comprehensive information now available on variation in the human genome, there are good opportunities to make significant progress toward identifying relevant risk alleles and to develop strategies that might enable at risk individuals to be identified while avoiding withdrawal of otherwise useful drugs.

Latin America is qualified to confront this ambitious project. We must now commit to hard work in order to achieve the aims. Working together with clear and effective strategies, we will accomplish our goals.

WHICH ARE THE FUTURE STRATEGIES FOR THE SPANISH-LATIN AMERICAN NETWORK TO PERFORM THE GOALS?

- To identify suspected cases of hepatotoxicity and to enter patients data in the ALEH website database.
- To perform thorough clinical data collection during a personal interview with the patient emphasizing the connection between drug treatments and liver disease. This investigation should discard herbs and other etiological agents. We must also carefully investigate the *start and end intake dates* of any drug involved in potential liver toxicity.
- To analyze every reported clinical case and identify causality with the suspected drug. The information will be entered in the ALEH section of hepatotoxicity and will be sent to the University of Malaga where three independent experts will review every case.
- The CIOMS scale will be applied to assign a numerical score, which is converted to a probability category, in every hepatotoxic reactions accepted by the expert committee. These protocols will then be stored in the database.
- To obtain biological samples from patients with toxic liver disease to be used for genetic and biomarkers studies. These studies could help us to clarify the pathogenic mechanism of liver damage.
- To create a biobank of biological samples.
- To analyze gathered information and present it in meetings and peer-reviewed journals.

Our goals will only be achieved if we can centralize patient management within the Spanish-Latin American Hepatotoxicity network using *consensus guidelines and uniform criteria*. Moreover, the creation of a biological sample bank and the possibility to access centers specialized in genotyping studies, will be an invaluable tool to enhance knowledge of the drugs causing hepatotoxicity.

The possibility of proving the association of a specific liver damage to genetic findings may ultimately lead to the development of new chemical structures replacing drugs that are commonly related to severe liver damage. Thus, increasing their safety profile as well as impacting on the future design of safer compounds. Achieving these goals, would not only enrich our epidemiological knowledge, but also improve the public health system.

ON BEHALF OF THE LATIN-AMERICAN DRUG-INDUCED LIVER INJURY NETWORK

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