

Sex differences in adverse drug reaction and liver disease

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ABSTRACT

Adverse drug reactions represent a significant public health problem. A share of no small adverse reaction is particularly serious and can even lead to patient's death. Furthermore, hepatitis induced by serious drug reactions is a rare event but potentially fatal. Finally, we know that epidemiological studies have highlighted male gender as an independent predictor of fibrosis progression towards cirrhosis in hepatitis B and C-virus, as well as non-alcoholic steatohepatitis. The importance of considering the differences between the male and female sex in clinical decision-making is crucial. The physiopathology of liver disease is different in the two sexes, but these differences are not yet fully known and several potential mechanisms have been identified.

Gender differences in pharmacological response: sex and adverse reactions

Adverse Drug Reactions represent a significant public health problem. Altogether, according to some authors, 5-10% of patients treated with drugs manifests an adverse reaction, about 5% of hospital admissions are due to adverse drugs reactions (ADRs), while the incidence of ADRs in hospital is more than 10%. A share of no small adverse reaction is particularly serious and can even lead to patient's death.^{1,2} The importance of considering the differences between males and females in clinical decision-making is crucial. Gender-oriented pharmacology is the branch of pharmacology that defines differences ef-

fectiveness and safety of drugs, possibly existing in men and women in order to get a safe and efficient evaluation of the treatment. The purpose is to obtain a personalized treatment *correct drug, patient and dosage*. As for reports of adverse drug reactions, women are more vulnerable than men. Female patients have a 1.5- to 1.7-fold greater risk of developing an ADR, including adverse skin reactions, compared with male patients. In Italy the data of the National Pharmacovigilance Network show a higher number (59% in 2011) of reported adverse drug reactions in female subjects than in men.

The greater severity and increased frequency of ADRs in women combine a certain number of factors such as: i) a particular female susceptibility (*e.g.*, fractures from thiazolidinediones, lupus erythematosus from procainamide, hydralazine, bleeding from thrombolytic agents, *etc.*); ii) polypharmacy (which is more common in women);³ iii) age (there are many more older women than older men);⁴ iv) the possibility of overdose; v) depression that is more common in women.⁵

Gender differences start in uterus and they can change during the life. Gender differences in pharmacology include the pharmacokinetics and pharmacodynamics difference among people using drugs. Different size, corporeal composition assimilation metabolism (phase 1 and 2) and the different elimination provides the basis for the pharmacokinetics sex differences. Renal clearance is generally higher in men that in women providing the basis for the pharmacodynamics sex differences. Despite this, the treatment revision compared to renal function and body weight is not a common activity. Effectively the suggested dose for most of drugs on the marketplace is calculated for a 70 kg man. The time of gastric emptying is affected by hormonal changes and it increases during pregnancy. For this reason, oral drug bioresource can be

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altered. An important parameter is women bodily composition with more adipose tissue than men, so distribution volume can decrease for hydrophilic drugs. Also, the pharmacokinetics drug standards depend on the effect of women hormonal changes that include estrogen and progesterone use for therapeutic and contraceptive purposes. The knowledge about *dynamic drugs* differences is scarce but it is renowned that women and men can have different drug targets. Even compared to organic systemic classes, with the exception of renal and urinary adverse reactions, which are more common in men, it is reported a higher number of reactions in women.

However, it has been acknowledged in recent decades that clinical trials have not always adequately enrolled women or analyzed sex-specific differences among the data. The enrollment of women in phase 1 and 2 clinical trials remains highly deficient.

It is also known, since 1980, that women bleed more in the course of heparin therapy with a higher frequency of thrombocytopenic purpura, a disease most frequently induced by heparin high molecular weight.⁶ Females' heart, then, is more sensitive to chemotherapy in general and not just to anthracyclines, developing more easily adverse events (*e.g.*, heart failure). For example, updated data to December 2015 show that the majority of ADRs collected for Bevacizumab are borne by women.⁷ It has been observed that, more than 100 molecules very heterogeneous among themselves within which the antiarrhythmics, antipsychotics, antidepressants, macrolide antibiotics (*e.g.*, erythromycin),azole antifungals, may prolong the QT interval in women, which sometimes can go to the *torsades de points*, a serious arrhythmia that can even be fatal. This depends on the fact that, after puberty, cardiac repolarization in females is longer than

about 20 msec.^{8,9} The sex-gender also influences the type of adverse reaction: for example, certain diuretics can cause hyponatremia in women and decreased plasma volume in men.¹⁰ With regard to the class of non-opioid analgesics, differences were ascertained with ibuprofen which, on equal plasma concentrations is more effective in men (Tables 1 and 2).¹¹ Lastly certain classes of drugs, including the current oral anticoagulants, were taken under examination and, an increased number of bone fractures in females was recorded in relation to them. In conclusion, despite the literature and signaling data suggest a higher frequency of adverse reactions in the female sex-gender, most of the available information is derived by post-hoc analysis, by meta-analysis of clinical studies and reports. Anyway, they have not considered the determining sex-gender in its complexity, thus including pharmacodynamic and hormonal changes, critical periods, lifestyle, and so on.

Sex differences in drug-induced liver injury

Hepatitis induced by serious drug reactions is a rare event but potentially fatal. The reported rate is between 1/10,000 and 1/100,000 patients. Approximately 20-30% of cases of acute liver failure, associated with a high degree of mortality, seems to be related to the use of medications.¹²

The drug-induced liver injury (DILI) is the most frequent cause of acute liver failure and liver transplantation in Western countries. The events range from a mild and asymptomatic increase in transaminases, which occurs with a relatively high frequency and with a high number of drugs, up to fulminant liver failure. The purpose of this paper is to focus on the art's

Table 1. Drugs and reactions in adverse drugs reactions.

Drug	Reaction	Total number (%) (N=164)
Ampicillin	Dizziness, rash, erythema, pruritus, diarrhea	18 (1.1)
Ciprofloxacin	Rash, chest pain, pruritus, dizziness, oral candidiasis	16 (9.8)
Nifedipine	Edema, burning sensations, palpitations, headache, restlessness	11 (6.7)
Cefotaxime	Diarrhea, rash, oral candidiasis	10 (6.1)
Diclofenac	Rash, shock, nausea/vomit	9 (5.5)
Atenolol	Fatigue, cough, edema	8 (4.9)
Theophylline	Tremor, supraventricular tachycardia, dizziness	8 (4.9)
Salbutamol	Palpitations, muscle cramps, tremors, dry mouth	7 (4.3)
Insulin	Burning sensation, rash, dizziness	6 (3.7)
Furosemide	Electrolyte imbalance, muscle cramps, gastritis	6 (3.7)
Metronidazole	Rash, melaena, diarrhea	6 (3.7)

Reproduced from Arulmani *et al.*, 2008.¹¹

state of the knowledge of biological mechanisms, risk factors, and diagnostic elements of the hepatic ADRs, through a systematic review of the literature.

The DILIs are classified as intrinsic or idiosyncratic ADRs. The hepatic intrinsic ADRs occur with short latency and have a high incidence in people taking high doses of the drug; these ADRs are not associated with hypersensitivity events. Idiosyncratic ADRs, instead, occur only in a minority of susceptible individuals, they have variable latency and they are not related to the action's mechanism of the drug. The main mechanisms of DILI are: i) the irreversible chemical modification of a protein that has an effect on its function; ii) formation of antigens; iii) irreversible chemical modification of DNA. Risk factors for DILI are childhood or advanced age, female sex, concomitant drug therapy, concomitant diseases, excessive alcohol consumption, malnutrition, underlying disease and genetic susceptibility. To establish a diagnosis of drug-induced liver injury is very difficult. Thus, after excluding other possible causes, it is important to identify a specific hepatic effect of one of the drugs taken by the patient. Some drugs that give hepatic ADRs are non-steroidal anti-inflammatory drugs (NSAIDs), troglitazone, acetaminophen, fluvastatin, isoniazid, flucloxacillin, ipilimumab, and pazopanib. To accurately detect early signs of liver damage, we need clinical biomarkers that are able to distinguish the drug-induced hepatotoxicity from other forms of liver injury and can differentiate mild hepatic lesions from those clinically important.

Most metabolic transformations of drugs, through the P-450 cytochrome, occur in liver. These enzyme systems are also subject to genetic polymorphism, making some patients particularly susceptible to drug interactions. Many drugs are lipophilic substances that are transformed into hydrophilic in the passage through the cytochrome P-450 with frequent formation of intermediate metabolites that are highly polar compounds with high reactivity. Advanced age, the pre-existence of liver disease, the induction/enzyme inhibition, genetic variants, but above all the intrinsic characteristics of the

molecule itself, affect the harmful event.^{13,14} The diagnosis of liver damage from drugs is often excluded. The chronological order is very important. Clinical criteria (exclusion of all other causes of liver injury, a history of multiple pharmacotherapy or taking medication known to hepatotoxicity) are also useful. Some scores and some tables on adverse reactions can help you decipher the suspicion.^{15,16}

The Naranjo's algorithm (Table 3) is a questionnaire designed by Naranjo *et al.* for determining the likelihood of whether an ADR is due to the drug or it is a result of other factors. Probability is assigned via a score termed definite (≥ 9), probable (5-8), possible (1-4) or doubtful (0).

Like other adverse reactions including those with liver load can be divided into predictable reactions, dose dependent, with high incidence (type A) and unpredictable reactions, dose-independent, with a low incidence (type B). A liver damage type A should be suspected if in a subject who started a new drug treatment in the last weeks or months are detected abnor-

Table 2. Systems and organs affected by adverse reactions.

Organ or system	Number (%) di ADR n=164
Skin	56 (34.1)
Central nervous system	31 (18.9)
Gastroenteric tract	29 (17.7)
Heart and vessels	28 (17.1)
Eye, ear, nose and throat	8 (4.9)
Muscles and skeleton	4 (2.4)
Metabolism	3 (1.8)
Blood	2 (1.2)
Genito-urinary tract	2 (1.2)
Respiratory system	1 (0.6)

ADR, adverse drugs reactions. Reproduced from Arulmani *et al.*, 2008.¹¹

Table 3. Modified Naranjo's algorithm.

Question	Yes	No	Do not know
Are there previous conclusive reports on this reaction?	+1	0	0
Did the adverse event appear when the suspected drug was administered?	+2	-1	0
Did the adverse reaction improve when the drug was discontinued or a specific antagonist was administered?	+1	0	0
Did the adverse reaction reappear when the drug was re-administered?	+2	-1	0
Are there alternative cause (other than the drug) that could on their own have caused the reaction?	-1	+2	0
Did the reaction reappear when a placebo was given?	-1	+1	0
Was the drug detected in the blood (or other fluids) in concentrations now to be toxic?	+1	0	0

malities in liver function tests, often without symptoms or with specific symptoms (nausea, dyspepsia, malaise). The type B is associated with the appearance of jaundice or with an increase in total bilirubin >3 mg/dL, with more than 50% of direct bilirubin. Jaundice can be isolated or associated to other symptoms (nausea, dyspepsia), and in certain cases, to extrahepatic manifestations (rash, lymphadenopathy, eosinophilia, thrombocytopenia, neutropenia, renal insufficiency with serum creatinine). Budd-Chiari syndrome is a rare presentation and is often related to the use of progesterone, usually in women with thrombophilic predisposition to hereditary causes (the most frequent is the factor V Leiden mutation) or acquired (most frequently myeloproliferative syndromes).¹⁷⁻²⁰ Therefore type B reactions may present with different clinical conditions: acute hepatocellular necrosis, acute hepatitis, steatosis, cholestasis with or without hepatitis, chronic active hepatitis, fibrosis and cirrhosis, chronic cholestasis, granulomatous hepatitis, Budd-Chiari syndrome, hepatic tumors.^{21,22}

There are three types of acute liver injury:

- *Hepatic*: prevailing increase in transaminases aspartate transaminase (AST) and/or alanine transaminase (ALT), with or without jaundice. The increase in transaminases AST and/or ALT can be moderate ($3.5 \times N$ or at least $>2 \times N$) or significant ($50-100 \times N$). It can coexist a modest increase in alkaline phosphatase. The acute liver injury is typically associated with isoniazid (which can also give chronic hepatitis), pyrazinamide, halothane, troglitazone.
- *Cholestatic*: increased alkaline phosphatase $>2 \times N$ with no increase or moderate increase of transaminases AST and/or ALT, and often increased gamma-glutamyl transferase and bilirubin. The cholestatic disease acute injury is typically associated with estrogen, tamoxifen, anabolic steroids, cyclosporine, azathioprine.
- *Mixed*: associated increase in transaminases AST and/or ALT and alkaline phosphatase, with or without jaundice. The Joint acute liver injury is typically associated with amoxicillin-clavulanic acid, tricyclic antidepressants, phenothiazines, NSAIDs.

Acute liver failure or mixed type can have serious characteristics and evolve into acute liver failure. Early severity criteria are: the presence and intensity of jaundice; the association of extrahepatic manifestations and, particularly, of serum creatinine; the rapid decrease of prothrombin. Thrombosis of the hepatic veins supra (Budd-Chiari syndrome) is the most severe form of acute liver injury. This clinical presentation requires immediate hospitalization in a well-equipped hospital where to place a transjugular portocaval shunt. After discontinuation of the drug this clinical pattern cannot regress and it can evolve in a syndrome of chronic portal hypertension.²²⁻²⁵ Parac-

etamol is the most common cause of ADR, followed by antibiotics, NSAIDs and anti-tuberculosis drugs.

The predominant factors determining liver injury differences between males and females are:

- *Difference in exposure to risk factors*: because of a slower metabolizing capacity and hormonal interference, women are mostly often involved in events by pharmacological interference. They are also exposed to gender-related elective treatments (progesterone contraceptives, hormone replacement therapy) that may cause alterations in the pharmacokinetics of other drugs taken simultaneously, or themselves be altered in their activity by other drugs (e.g., increased likelihood of unwanted pregnancies for interaction between progesterone and carbamazepine).^{22,23} Moreover, in recent years alcoholism has become much more common among Western women and, in parallel, there has been an increase of alcohol-related problems. It was shown that, women develop more severe pathologies in a shorter time (telescoping effect).²⁶⁻²⁸
- *Protective effect/aggravation of sex hormones*: it is assumed that the gonadic hormones exert their effects on the metabolism of drugs acting directly on the liver.²⁹⁻³¹ It is known that the metabolism of drugs in the liver is regulated by the expression of so-called *major drug-metabolizing enzymes* that include the P-450 cytochrome, sulfotransferase, glutathione transferase and uridildiphosphate-glucuroniltransferase.³¹⁻³⁶ Sex hormones influence the bioavailability of drugs taken orally having modulating effects on motility and so on gastrointestinal transit. The estrogens, for example, inhibit gastric emptying.³¹ The hormonal fluctuations also affect the bioavailability of the drugs. Body weight (usually lower in women), body fat (usually higher in women), the plasma volume (lower in women, but with wide variations in menstruation and pregnancy), and blood flowing of the principal organs (higher in women) may result in different efficacy or different risk of side effects than the female counterpart.^{31,36} In women with a story of abuse or alcohol use, estrogen and progesterone may affect the gastric and liver alcohol dehydrogenase activities, making women more susceptible to drugs damage.³⁷⁻⁴⁰
- *Differences related to genetics*: we have already mentioned how some hormones and particularly growth hormone (GH)^{41,42} are capable of modulating the transduction and gene transcription of some of the major human hepatic enzymes involved in drug metabolism. Indeed CYP3A4, the most important among catalyst molecules of cytochrome P450 is expressed more in women, and this expression is strictly related to high expression of mRNA encoding.⁴³ Even a high activity of CYP2B6 was found

in women and this in parallel with an increase in the expression of its gene.⁴⁴ Furthermore in the human *CYP3A4* gene, there are many gene sequences that are sensitive to the endogenous hormonal environment such as plasma values of GH.³¹ GH secretion continues, takes part in the activation of specific female genes CYP3A family by converting to nuclear heterochromatin level (non-coding portion of DNA) in euchromatin (DNA portion coding) that in the case of some transcription factors activate liver cells for *P450* gene expression.^{45,46}

- *Difference related to the body structure*: body structure mainly modifies the bioavailability of the drug in women compared to their male counterparts, since it depends largely on the distribution of body fat and lean body mass, fluid distribution between circulating volume and third space blood supply of the organs.³¹ This may in turn affect the bioavailability of drugs and lead to delays from the point of view of both the pharmacokinetic or pharmacodynamic drug metabolism activity that distinctly lipophilic to hydrophilic women compared to their male counterparts.⁴⁷

Liver disease in women: the influence of gender

The physiopathology of liver disease is different in the two sexes, but these differences are not yet fully known and several potential mechanisms have been identified, including: i) the effects of sex hormones on liver metabolism and oxidative stress;⁴⁸⁻⁵¹ ii) differences of cytochrome P450, glucose 6-phosphatase and glutamine synthetase;^{52,53} iii) estrogens influence the levels of steroid binding globulin, angiotensinogen, ceruloplasmin and transport proteins;⁵⁴ iv) a gender dimorphic pattern of response in gene transcription in the pathological stress (like in an ischemia/reperfusion injury a different response was observed in female and male liver);⁵⁵ v) different response of the immune system in women than in men.

Except for autoimmune diseases, hepatic fibrosis is largely male dominant.⁵⁶

Epidemiological studies have highlighted male gender as an independent predictor of fibrosis progression towards cirrhosis in hepatitis B and C (HCV) viruses, as well as non-alcoholic steatohepatitis (NASH);⁵⁷⁻⁵⁹ these data were confirmed by experimental studies on rats.^{60,61} Nevertheless, gender differences in the healthy liver are much more obscure, in rats as in humans. Even if the collagen content of the liver is much lower than in any other organ, significant gender differences in rats have been found: 2.5 *versus* 1.9% in males and females, respectively.⁶² Therefore, it is reasonable to hypothesize that such differences may also apply for humans, since studies using transient

elastography (Fibroscan) in healthy patients have shown significant differences, pointing at greater extracellular matrix content in the male liver.⁶³⁻⁶⁵ In this vein, it may be argued that before the onset of fibrosis (NASH or HCV related), men would already have more fibrous tissue and an increased risk of severe liver fibrosis.⁶² It has long been known that proliferation of Kupffer cells (KC), as well as peaks of their phagocytic activity, are correlated with raised estrogen levels in the estrous cycle of rodents;^{66,67} for instance, ethinyl estradiol (a major component of several combined oral contraceptive pills) induces a fivefold increase in KC proliferation *in vitro*.⁶² According to data of rats, estrogens also influence the normal liver, since the numerical density and number per gram of KC differ across genders, especially among younger animals.⁶⁸ Some have also shown that in female Wistar rats there is an increased number of macrophages in pleural and peritoneal cavities with enhanced phagocytic activity than males.⁶⁹ Besides numerical differences, hormones are also relevant: estrogens, for instance, exert anti-inflammatory and anti-oxidative actions, by inhibiting the production of pro-inflammatory tumor necrosis factor- α (TNF- α), interleukin-1b and -6.⁷⁰ Accordingly, the menopause is associated with spontaneous increases in the above-mentioned cytokines in women.⁷¹

Another functional consequence of KC dimorphism resides in alcohol susceptibility, which is greater in female (rats as well as humans).^{72,73} Using an enteric feeding model, it was shown that young female rats had an increased pathology score, more marked infiltration by neutrophils and higher endotoxin levels, which ultimately was responsible for a stronger activation of KC, when compared with males; moreover, female KC had an increased production of TNF- α and reactive oxygen species.⁷²⁻⁷⁴ The liver is singular in this regard, as it is the only organ which, after being reduced to a third, is capable of an organized tissue growth to regain its original weight, with a fairly high precision (less than 10% variation). After partial hepatectomy, quiescent HEP start to replicate, therefore restoring the functional liver tissue. A contribution for this is achieved by BnHE, acting as an important cell reservoir that rapidly generates mononuclear HEP by amitotic cytokinesis.⁷⁵ Recently, it was shown that female rats have higher hepatocellularity with a larger proportion of BnHEP.⁷⁶

We hypothesize that the same occurs in women, resulting in a higher regenerative potential as it is shown by some studies on rats,⁷⁷⁻⁷⁹ and humans,⁸⁰ although there are very few data.

It is known that endogenous estrogens increase after partial hepatectomy, eliciting a response by HEP (more than in KC): a rapid translocation of the estrogen receptor from the cytoplasm to the nuclei occurs

and DNA synthesis is increased.^{81,67} Notably, a short-term adjuvant therapy of estrogen has already been proposed for promoting liver regeneration after partial hepatectomy, in patients with poor liver function.⁸² Currently there are controversial data on the action of estrogens: in particular studies in rats after ovariectomy have come to conflicting conclusions.⁸³⁻⁸⁵ Apart from differences in regeneration, the higher hepatocellularity of female rats corroborates the larger functional reserve for this gender. In fact, it is nowadays recommended to use different normal reference levels for aminotransferase activity in men and women.⁸⁶

The consequences of the gender dimorphism in liver structure and cell composition encompass liver fibrosis, alcoholic injury and post-hepatectomy regeneration, thus sustaining the concept of gender specific hepatology. Another consequence of our hypothesis resides in liver transplantation, since structural dimorphism may help explain gender-mismatch liver transplantation. It may be hypothesized that when deprived with of estrogenic milieu (inherent to transplantation in a male recipient), the highly hepatocellular female liver may start remodeling and the HEP apoptosis may trigger an increased production of pro-inflammatory interleukins (by more numerous KC population). Eventually, this may sentence the female organ to a poorer outcome in the male recipient.

The functional significance of differences in HEP, KC and in collagen disclosed herein are still poorly understood. In conclusion, liver gender dimorphism extends from genes and enzymatic activities up to the morphological level, at least in the rat.

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