Incidence and Severity of Drug-Induced Acute Liver Injury Following Bone-Marrow Transplantation

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List of abbreviations

(D)	Donor
(R)	Recipient
µmol/L	Micromole per litre
ADL	Activities of Daily Living
ADRs	Adverse drug reactions
AE	Adverse event
AldoCY	Aldocyclophosphamide
Alk	Alkeran
ALL	Acute lymphoblastic leukemia
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AML	Acute myeloid leukemia
AST	Aspartate aminotransferase
ATG	Antithymocyte globulin
ATP	Adenosine triphosphate
BCG	Bacillus-Calmette-Guerin
BCNU	Carmustine (bis-chloroethylnitrosourea)
BMT	Bone marrow transplantation
BTM	Beta thalassemia major
BU	Busulfan
C.M.V.	Cyclophosphamide, melphalan (alkeran), etoposide.
CAA	Chloroacetaldehyde
CBC	Complete blood count
CD	Cluster of differentiation
CEPM	O-carboxyethyl-phosphoramide mustard
Cgy	Centigray
CIOMS	Council for International Organizations of Medical Sciences
CML	Chronic myeloid leukemia
CMV	Cytomegalovirus
CSA	Cyclosporine
CTCAE	Common Terminology Criteria for Adverse Events
CY	Cyclophosphamide
CYP	Cytochrome P
DCCY	Deschloroethyl-cyclophosphamide
DD	Death domain.
DILI	Drug induced liver injury
DILIN	Drug induced liver injury network
DNA	Deoxyribonucleic acid
EBV	Epstein- Barr virus
Flu	Fludarabine

GSCY	Glutathionyl-cyclophosphamide
GVHD	Graft versus-host disease
Gy	Gray
HBc Ab	Hepatitis B virus core antibody
HBs Ab	Hepatitis B virus surface antibody
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCV Ab	Hepatitis C virus antibody
HCY	4-hydroxycyclophosphamide
HHV-6	Human Herpesvirus 6
HHV-8	Human Herpesvirus 8 (Kaposi's sarcoma-associated Herpesvirus)
HLA	Human leukocyte antigen
HPPM	Hydroxypropyl-phosphoramide mustard
HSCT	Hematopoietic stem cell transplantation
HSV	*Herpes simplex* virus
i.v.	Intravenous
IFN	Interferon
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-6	Interleukin-6
INH	Isoniazide
LFTs	Liver function tests
LPD	Lymphoproliferative disease
Max	Maximum
MDS	Myelodysplastic syndrome
Melph	Melphalan
mg/dl	Milligrams per decilitre
Min	Minimum
MM	Multiple myeloma
MRP3	Multidrug-resistance–associated protein 3
N	Number
NHL	Non-Hodgkin's lymphoma
NSAIDs	Non-steroidal anti-inflammatory drugs
PBSCs	Peripheral blood progenitor cells
PCR	Polymerase chain reaction
PDR	Physicians' desk reference
PM	Phosphoramide mustard
R	The ratio of serum activity of ALT/serum activity of alkaline phosphatase (ALP)
RNA	Ribonucleic acid
RUCAM	Roussel-Uclaf Causality Assessment Method
SAA	Severe aplastic anemia
SOS	Sinusoidal obstruction syndrome

SPSS	Statistical Package for Social Sciences
TBI	Total body irradiation
TNFα	Tumor necrosis factor α
Tox	Toxoplasma
UCB	Umbilical cord blood
ULN	Upper limit of normal
VOD	Veno-occlusive disease
VZV	*Varicella-zoster* virus
Vp-16	Etoposide
WNL	Within normal level

Abstract

Background: In no other medical situation is a patient at risk for so many liver diseases as during a hematopoietic stem cell transplant (HSCT).

Aim: Evaluation of the incidence, causality and severity of drug-induced liver injury (DILI) in HSCT recipients.

Patients and methods: All patients post-HSCT presenting to the Nasser Institute were included in the study and those with any other cause of liver injury, were excluded. Liver Function Tests (LFTs) monitoring was used for assessment of liver diseases. The type and severity of DILI was determined based on the pattern and degree of LFTs elevation. The use of the Roussel-Uclaf Causality Assessment Method (RUCAM) confirmed the determined causality of DILI.

Results: Out of the 132 HSCT patients assessed; 112 (84.8%) were allogenic and 20 (15.2%) were autologous. In allogenic patients, the cumulative incidence of DILI till the end of each of the early, intermediate and late intervals was 27 %, 37.8 % and 46.3 % respectively. There was no significant difference in the incidence of DILI with regards to sex, conditioning regimen category, age, diagnosis category, abnormal chemical and virological parameters pre-transplantation. Causes of DILI were: conditioning regimen (55 %), cyclosporine (40 %) or fluconazole (5%) and the types of injury were; hepatocellular(95%), cholestatic (2.5%) and mixed (2.5%). Injury severity included patients with ; severity grade 1(30 %), grade 2 (37.5%) and grade 3(32.5%). One patient developed DILI in autologous patients group.

Conclusion: There was no significant difference in the incidence of DILI over the 3 intervals with regards to the different variables. Most cases with DILI post-HSCT were hepatocellular. Conditioning regimen-induced injuries were more severe than CSA-induced.

Key words: Hematopoietic stem cell transplantation (HSCT), drug-induced liver injury (DILI).

Introduction

Liver injury can be caused by any drug, even the safest ones (Galan *et al.*, 2005).One-half of the medications listed in the Physicians' Desk Reference (PDR) are associated with some degree of liver injury, and 100 of these are reported to be capable of causing fulminant hepatic failure (Lewis, 2002).

Drug Induced Liver Injury (DILI) accounts for roughly 0.1% to 3% of hospital admissions, 600 liver transplantations, and 120 deaths from liver failure in the United States (US) each year (Lazerow *et al.*, 2005; Lee and Senior, 2005). DILI is one of the leading causes of acute liver failure in the US, accounting for 13% of cases of acute liver failure (Suk and Kim, 2012).

Worldwide, the estimated annual incidence rate of DILI is 13.9-24.0 per 100,000 Inhabitants (Suk and Kim, 2012).DILI, is also an important concern for pharmaceutical companies as it can lead to drug withdrawal after marketing, or during phase II or III clinical trials (Fromenty, 2013).

Mainly, drugs tend to induce acute hepatitis, cholestasis or a mixed condition, because each hepatocyte may be the target of drug-induced toxicity, many other expressions of hepatotoxicity may be evident, including chronic hepatitis, cirrhosis, sinusoidal obstruction syndrome or neoplasm (Zimmerman, 1999).

Drug induced liver injury spans the entire spectrum ranging from asymptomatic elevation in transaminases to severe disease such as acute hepatitis leading to acute liver failure (Devarbhavi, 2012).

Liver histology is the ideal tool for defining the pattern of hepatotoxicity. However, since a liver biopsy specimen is often not available, the pattern of DILI is, from a practical standpoint, classified according to laboratory data. This mainly includes the activity of serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) with the increase in activity being expressed with respect to the upper limit of normal (ULN) and the ratio of the measured activities(R) (Benichou, 1990).

The liver specific Roussel-Uclaf Causality Assessment Method (RUCAM) is the most validated and extensively used for determining the likelihood that an implicated drug caused DILI (Devarbhavi, 2012).

Patients are at the highest risk for so many liver diseases during a hematopoietic stem cell transplantation (HSCT), than any other medical situation (Shulman and McDonald, 2007).Severe liver damage is a major concern in patients undergoing HSCT with mortality rates of 4–15%. The most frequent causes are sinusoidal obstruction syndrome (SOS), liver graft versus-host disease (GVHD), infections due to various bacterial, viral and fungal agents, drug toxicity, total parenteral nutrition, and liver involvement by the initial malignancy (El-Sayed *et al.*, 2004).

In Egypt, two problems further complicate the situation of liver injuries after HSCT, these are schistosomiasis and hepatitis as the population of Egypt has a heavy burden of liver diseases (Mahmoud *et al.*, 2008).

Polypharmacy is a necessity post-transplant, with most patients receiving drugs for prophylaxis against infection (usually acyclovir, fluconazole, and trimethoprim-sulfamethoxazole combination), GVHD prophylaxis (usually tacrolimus or cyclosporine plus methotrexate or mycophenolatemofetil), antiemetics, antihypertensives, and ursodiol (Shulman and McDonald, 2007).

Hence, early detection, proper DILI diagnosis and management are of great importance, in order to minimize the hepatotoxicity complications, improve quality of life, decrease mortality rates and decrease the financial burden due to such complications post-transplant.

I. Drug-induced liver injury (DILI)

Drug-induced liver injury is defined as a liver injury caused by various medications, herbs, or other xenobiotics, leading to abnormalities in liver tests or liver dysfunction with the reasonable exclusion of other etiologies (Suk and Kim, 2012).

Essentially, any drug, even the safest ones, can cause liver injury (Galan *et al.*, 2005).One-half of the medications listed in the Physicians' Desk Reference (PDR) are associated with some degree of liver injury, and 100 of these are reported to be capable of causing fulminant hepatic failure (Lewis, 2002). Acetaminophen is the most common cause of DILI followed by antibiotics, NSAIDs, amiodarone, and anti-tuberculosis medications (Aithal and Day, 1999; Hartleb *et al.*, 2002; Galan *et al.*, 2005).

The impact of DILI on the pharmaceutical industry has led regulatory agencies to restrict the use of certain medications, issue black box warnings and even withdraw drug from the market (Lewis, 2000). DILI has been estimated to be the most frequent cause of medication withdrawal (Ostapowicz *et al.*, 2002; Lee, 2003; Wei *et al.*, 2007). Examples in the US and Europe are troglitazone, bromfenac, trovafloxacin, ebrotidine, nimesulide, nefazodone and ximelagatran (Shah, 1999; Lee, 2003; Mohapatra *et al.*, 2005).

A. Epidemiology:

There are limited data regarding the incidence of DILI because most mild cases of DILI do not get reported. Studies have shown that DILI is responsible for approximately 30% of cases of acute hepatitis referred for liver disease evaluation (Galan *et al.*, 2005).

Drug-induced liver injury has been estimated to account for roughly 0.1% to 3% of hospital admissions, 600 liver transplantations, and 120 deaths from liver failure in the United States each year (Lazerow *et al.*, 2005; Lee and Senior, 2005), and was estimated to be the number one cause of death from acute liver failure in U.S. (Lazerow *et al.*, 2005).

The estimated frequency of DILI for any particular medication varied from 1 in 1,000 to 1 in 100,000 patients (Bussieres and Habra, 1995; Lee, 2003; Rashid *et al.*, 2004). Acetaminophen alone was responsible for 50% of cases of acute liver failure in the U.S.(Lee and Senior, 2005) and was estimated to be the leading factor for liver transplantation (Russo *et al.*, 2004).
The most frequently implicated drugs after acetaminophen were isoniazid, prophylthiouracil, phenytoin and valproate (Russo *et al.*, 2004).

B. Pathophysiologic mechanisms of DILI:

At least six mechanisms that primarily involve the hepatocyte produce liver injury, and the manner in which various intracellular organelles are affected defines the pattern of disease Figure 1.

If high-energy reactions involving cytochrome P-450 enzymes lead to covalent binding of drug to intracellular proteins, intracellular dysfunction is apparently produced that results in the loss of ionic gradients, a decline in ATP levels, and actin disruption, cell swelling, and cell rupture (Figure 1A) (Beaune *et al.*, 1987; Yun *et al.*, 1993).

Drugs that affect transport proteins at the canalicular membrane can interrupt bile flow. Certain drugs, for example, bind to or disable the bile salt export protein. This process causes cholestasis; however, little cell injury occurs (Figure 1B) (Trauner *et al.*, 1998).

Genetic defects in transporters, as in the multidrug-resistance–associated protein 3 (MRP3), in combination with hormones may promote cholestasis during pregnancy or during treatment with estrogen-containing medications. In mixed forms of hepatic injury, the combined failure of canalicular pumps and other intracellular processes allows toxic bile acids to accumulate, causing secondary injury to hepatocytes. If cells of the bile ducts are injured, a likely outcome is protracted or permanent cholestasis, a disorder that has been termed the “vanishing bile duct syndrome” (Lee, 2003).

Drugs are relatively small molecules and, therefore, are unlikely to evoke an immune response. However, biotransformation involving high-energy reactions can result in the formation of adducts; that is, drugs covalently bound to enzymes. Adducts that are large enough to serve as

immune targets may migrate to the surface of the hepatocyte, where they can induce the formation of antibodies (antibody- mediated cytotoxicity) or induce direct cytolytic T-cell responses (Figure 1C and 2D) (Robin *et al.*, 1997).

The secondary cytokine is then evoked and may cause inflammation and additional neutrophil-mediated hepatotoxicity (Jaeschke *et al.*, 2002). Programmed cell death (apoptosis) can occur in concert with immune-mediated injury, destroying hepatocytes by way of the tumor necrosis factor (TNF) and the Fas pathways, with cell shrinkage and fragmentation of nuclear chromatin (Figure 1E) (Reed, 2001). Pro-apoptotic receptor enzymes, if activated by drugs, will compete with protective so-called survival pathways within the cell, and this dynamic interaction may shift the balance either in favor of or against further cell damage.

Still other pathways to injury may develop when drugs damage mitochondria, disrupting fatty-acid oxidation and energy production. When drugs bind to or otherwise disable respiratory-chain enzymes or mitochondrial DNA, oxidative stress results, with ensuing anaerobic metabolism, lactic acidosis, and triglyceride accumulation (micro-vesicular fat within cells) (Figure 1F) (Pessayre *et al.*, 2001). Steatohepatitis (fat that primarily accumulates in the large vesicles outside the liver cells, with associated inflammation) is commonly associated with alcohol abuse, but it may also result from drugs (Lee, 2003).

Other cells within the liver may be the target of drug injury or serve as modulators of an incipient reaction. For example, Kupffer cells activate cytokines that may amplify injury (Jonsson *et al.*, 2000) and fat-storage cells (stellate cells), or macrophages may augment injury, produce fibrosis, or form granulomas. Hemotherapeutic agents can injure sinusoidal endothelial cells, a process that can lead to veno-occlusive disease (VOD) (DeLeve *et al.*, 2002). Therapeutic hormone administration may induce hepatocyte dedifferentiation, resulting in benign adenomas and, rarely, carcinomas. Clearly, multiple cellular pathways to liver injury are possible (Lee, 2003).