Drug-induced hepatitis – morphological and ultrastructural aspects

AURELIA ENESCU1), P. MITRUT2), ELENA BUTEICĂ3), B. STĂNOIU4), ANCA ENESCU5)

1)Medical Emergency Clinic
2)Internal Medicine Clinic
3)Genetics Department
4)Cellular and Molecular Biology Department
5)student

University of Medicine and Pharmacy of Craiova

Abstract
Frequency of drug-induced liver diseases is increasingly, more than 200 different drugs being incriminated in hepatic disorders. We performed a retrospective study on 65 cases of deaths due to drug intoxications and an experimental model of subacute hepatitis induced by acetaminophen. In our experimental model, we observed various histological lesions: granular degeneration, vascular congestion, lymphoplasmocyte infiltration, cytonecrosis. Histological criteria are not specifically, but very usefully, because based on these criteria we can suspect a drug etiology in hepatic disorders when any other cause is absent. Ultrastructural study of hepatocytes revealed some modifications, in addition to data provided by optical microscopy.

Keywords: drug-induced hepatitis, acetaminophen, ultrastructural study.

Introduction
Liver is the central organ not only for drugs metabolism, but also for toxic reactions [1].

All statistics from the last decade notice a marked increase of secondary drug – reactions, due to the huge number of drugs added to an impressive list of hepatotoxic drugs [2].

Hepatic reaction to some drugs depends on interactions between absorption, administration conditions and genetic factors.

Importance of drug-induced hepatic lesions is a consequence of frequency and difficulties in differential diagnosis, and results also from pathological mechanism and clinical evolution [3].

Frequency of hepatic disorders drug-induced is definitely increasing [4].

Women present a higher frequency than men and the maximum incidence is in patients after 40 years old.

Moreover, the gravity of secondary drug – induced hepatic reactions increased as importance and about 14% are vitally [5].

More than 200 different drugs are incriminated in hepatic disorders. It is necessary especially a longer observation of drugs after commercialization, a regular report of secondary drug – reactions and a right information of the physician.

Material and methods
We performed:
• A retrospective study (between 2002–2007) on 65 cases dead by drug-induced intoxications, based on the data provided by Forensic Medicine Laboratory.
• An experimental model of acetaminophen-induced subacute toxic hepatitis, considering the large utilization of this drug, with or without medical prescription. The experimental protocol consists in administration of 1 ml dose of acetaminophen 3%, hypodermically inoculated to a group of 25 Wistar rats. The sacrifices have been done after 2, 3 and 4 weeks.

Results
We performed anatomo-pathological examination on 65 dead patients, 38 being women and 27 men. Distribution of cases on age periods was as the following:
• 0–30 years: 13 cases;
• 30–40 years: eight cases;
• 40–50 years: 18 cases;
• >50 years: 26 cases.

Death occurred due to cardio-respiratory stop, acute hepato-renal insufficiency.

We present some significant cases, focusing especially on microscopic aspect of the liver.

Case no. 1
• F. S., male, 43-years-old.
• Diagnosis: acute barbiturate intoxication.
• Anatomo-pathological examination:
  – liver: sinusoidal stasis, stasis in centrolobular vein;
– lung: hyperemia, edema, emphysema;
– kidney: glomerular corpuscles with ischemic aspect, necrosis in tubular epithelium.

**Case no. 2**
- A. I., female, 23-years-old.
- Diagnosis: acute respiratory failure, acute drug intoxication, manic-depressive psychosis (Ludomil therapy), coma grade III.

**Anatomo-pathological examination:**
– liver: stasis in centrolobular vein, sinusoidal stasis;
– lung: edema, hemosiderin-containing macrophages in alveolar lumen;
– kidney: glomerular ischemia, tubes with atrophic epithelium and lacking nuclei.

**Case no. 3**
- C. F., female, 52-years-old.
- Diagnosis: acute drug intoxication, coma grade III.

**Anatomo-pathological examination:**
– liver: portal space fibrosis, panlobular microvesicular steatosis, biliary stasis, mild intralobular inflammatory infiltrate;
– kidney: chronic pyelonephritis.

**Case no. 4**
- N. I., female, 25-years-old.
- Diagnosis: acute drug intoxication

**Anatomo-pathological examination:**
– liver: portal space stasis, granular and vacuolar degeneration of hepatocytes;
– lung: edema, hemosiderin-containing macrophages in alveolar lumen;
– kidney: glomerular corpuscles with ischemic aspect, tubes with atrophic epithelium and lacking nuclei.

**Case no. 5**
- B. F., male, 52-years-old.
- Diagnosis: acute hepatorenal failure, acute intoxication with unknown agent.

**Anatomo-pathological examination:**
– liver: panlobular large coagulative necrosis, keeping lobular architecture;
– lung: scleroemphysema, zonal atelectasis;
– kidney: nephroangiosclerosis, acute tubular necrosis.

Histological changes found in forensic medical cases with anatomo-pathological examination have been variously.

We observed biliary stasis, dilatation of centrolobular vein, central/mediolobular micro/macroversicular steatosis, inflammatory infiltrate in portal space, fibrosis, granular and vacuolar degeneration, centrolobular necrosis (Figures 1–4).

Study of histological samples from the animals treated with acetaminophen 3% revealed significant changes of hepatic parenchyma and stroma.

In animals sacrificed after two weeks from toxic administration, lesions of hepatic parenchyma and stroma are moderately. Thus, we observed granular degeneration, vascular congestion in sinusoidal capillary, congestion in Kiernan space, perivascular edema, broken reticulin fibres (Figures 5 and 6).

In animals sacrificed after three weeks from acetaminophen 3% solution administration we found an increase of granular degeneration lesions, lymphoplasmocyte infiltrate, dilated sinusoidal capillaries (Figures 7 and 8).

In animals treated with acetaminophen 3% and sacrificed after four weeks, we found most noticeable changes. Thus, using silver staining we revealed important lesions of hepatic dystrophy, with a deeply modified fibrillary structure. Using Hematoxylin–Eosin staining, we evidenced granular degeneration and cytonecrosis lesions (Figure 9).

Ultrastructural study of hepatocytes revealed some modifications to complete the information obtained by optical microscopy. Thus, we observed hepatocytes having deep disorders in all cytosolic compartments, with vacuoles and abundant polyriboosomes (Figure 10).

**5 Discussions**

Histological criteria are not specifically and cannot identify certainly the drug etiology of hepatic disorders. However, some histological features could suggest a possible drug etiology and could be a relevant support for etiologic diagnosis. Because histological criteria are not absolutely, it is necessary to be interpreted in the context of clinical data and paraclinical investigations (International Group Lancet, 1974).

Histological criteria include, in the first time, zonal aspects of hepatocellular necrosis, with dominant centrolobular lesions and massive cell necrosis, disproportionately comparing the clinical and biochemical features. The same significance has the existence of more different necrosis points [6].

The dominance of eosinophils in portal and lobular inflammatory infiltrate has diagnostic value, if other cause, like parasitic disease, was excluded. Isolated cholestasis, especially centrolobular one, sometimes with lesions of biliary ducts epithelium, and periportal cholestasis early installed in disease evolution suggest the possibility of drug etiology.

Histological criteria are not specifically, because they appear in other circumstances too. However, they are very useful, because if other cause is excluded, they suggest the drug etiology for hepatic lesions and contribute to the right diagnosis [7].

Our results confirm the toxicity of acetaminophen and its potential to induce a toxic hepatic disorder.

Acetaminophen is extensively used in medicine due to its analgesic and antipyretic properties, and sometimes is used for suicide. Its presentation form is like white or light yellow crystal powder, with bitter taste and low soluble in water [8].
Figure 1 – Stasis in portal space; granular and vacuolar degeneration of hepatocytes (HE staining, ×200)

Figure 2 – Central/mediolobular micro/macrovesicular steatosis. Inflammatory infiltrate in portal space (HE staining, ×100)

Figure 3 – Portal space fibrosis; panlobular microvesicular steatosis, biliary stasis, mild inflammatory infiltrate; biliary thrombi, biliary pigment inside the hepatocytes (HE staining, ×200)

Figure 4 – Biliary stasis in hepatocytes, vacuolar and granular degeneration (HE staining, ×200)

Figure 5 – Acetaminophen treatment, granular degeneration (HE staining, ×200)
Figure 6 – Kiernan’s space congestion, mild lymphocyte infiltrate (Paracetamol treatment) 
(HE staining, ×200)

Figure 7 – Intensive granular degeneration; perilobular vein congestion (after three weeks from acetaminophen administration) 
(HE staining, ×200)

Figure 8 – Granular degeneration, intensive karyopyknosis, early periportal fibrillogenesis, severe stasis, perilobular vein congestion 
(Trichromic GS staining, ×400)

Figure 9 – Granular degeneration, hepatocytonecrosis 
(HE staining, ×400)

Figure 10 – Deep hepatocyte disorders in all cytosolic compartments
The severity of hepatic lesions is correlated with acetaminophen blood level, more than 300 µg/ml after four hours from ingestion being predictive for serious hepatic lesions, while under 150 µg/ml hepatic disorder is improbable or a mild one [9].

Hepatic disorder can be increase in case of administration together with alcohol or other agents that stimulate oxidase system and also in case of coexistence of some factors that reduce the hepatic level of glutathione (antecedent hepatic disease, malnutrition) [10].

Newman TJ and Bargman GJ reported the existence of more factors, which can modify the acetaminophen mechanism, such as preliminary treatment with Phenobarbital [11].

It has been suggested also that malnutrition predisposes to more severe hepatic lesions, following the acetaminophen overdose [12].

Alcoholic people have an increased risk to develop acetaminophen intoxication. Molecular base for high susceptibility of these patients to acetaminophen intoxication is unknown, but there are two theories: one is that alcoholic liver is empty of glutathione and unable to detoxify electrophilic molecules or acetaminophen metabolites; the second is that ethanol consumption stimulates or induces P450–11–E, which seems to generate toxic metabolites.

Acetaminophen, according to British Medical Journal (no. 7146), is the most frequent cause of acute hepatic failure, registering more than half from all cases.

Acetaminophen administration to test animals induced important modifications of hepatic parenchyma and stroma. It is well-known that acetaminophen can determine necrosis, especially in IIIa zone, inflammatory infiltrate [13, 14].

In our experiment, following the administration of a 3% concentrated solution of acetaminophen has been revealed various histological lesions. After two weeks from toxic administration, we noticed granular degeneration modifications, vascular congestion in sinusoid capillaries, perivascular edema, breaking down of the reticulin fibers.

These lesions were present also in histological samples gathered after three weeks, but in this case were more extensively and supplementary got vascular degeneration lesions, perilobular fibrillogenesis and mild lympho-plasmocyte infiltrate.

After for weeks after toxic administration we noticed a large diversity of lesions. Hepatic lobule has a disordered architecture due to enlarge of the sinusoidal capillaries, Disse spaces and space between hepatocytes.

We observed degenerative lesions of parenchyma, with some hepatocytes presenting eosinophilic intracyto-plasmatic material.

We also found granular degeneration and cytonecrosis lesions, lympho-plasmocyte infiltrate.

The silver staining revealed extended lesions of hepatic dystrophy, with a deep modified fibrilar apparatus.

Ultrastructural study of hepatocytes evidenced some changes, completing the information provided by optical microscopy.

Conclusions

Drug etiology has to be excluded for any chronic hepatitis.

Any adverse drug reaction is mandatory to maintain medical attention.

Anatomico-pathological examination in deadly cases revealed various lesions: granular and vacuolar degeneration, inflammatory infiltrate in portal space, sinusoidal stasis, hepatocytes necrosis.

In the experimental model used, Paracetamol induced mild hepatocellular necrotic lesions, visibly especially in the IIIa zone, low inflammatory reaction and periportal fibrosis.

Disorder of lobular architecture due to enlarge of the sinusoidal capillaries, Disse spaces and space between hepatocytes has developed in the same time with hepatocellular lesions and inflammatory reaction.

Electron microscopy has shown severe hepatocytes lesions, break down of smooth and rough endoplasmatic reticulum, mitochondria with larger volume and strange form, constant lack of glycogen, cytosolic lipid inclusions, diluted sinuoids with diffuse zonal fibrosis in Disse spaces, diffuse fibrosis surrounding hepatocytes.

References


Corresponding author
Aurelia Enescu, Associate Professor, MD, PhD, Medical Emergency Clinic, University of Medicine and Pharmacy, 2–4 Petru Rareş Street, 200 349 Craiova, Romania; Phone +40723–415 938, E-mail: ancaenescus@yahoo.com

Received: October 20th, 2007

Accepted: November 25th, 2007