THE MECHANISMS OF LIVER TOXICITY INDUCED BY ORGANIC SOLVENTS AND OTHER CHEMICAL COMPOUNDS

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Abstract: *Occupational exposure to organic solvents and other chemicals often induces non-specific liver damage. Therefore, although the number of chemical hazards present in the workplace is high, the percentage of liver disease diagnosed or suspected to be work-related is rather low. This can be explained by the fact that, aside from some specific compounds, liver damage induced by exposures in occupational setting is like the one induced by other toxic substances. This review focuses on the mechanisms of liver toxicity initiated by occupational chemical hazards, detailing the conditions for toxicity, the role of structural topographical units, lesion determinations and pathogenic pathways, while also mentioning examples of workplaces that are at risk.*

INTRODUCTION

Chemical industry has known a development without precedent during the last century, generating numerous organic and inorganic compounds in the environment of man, which has become a "chemical jungle".(1) Although the liver is one of the main targets of organic solvents, occupational-induced liver disease is rarely suspected and diagnosed in current medical practice.(2) This undervaluation is partly because, aside from specific lesions induced by some compounds, liver damage produced by occupational exposure does not differ clinically or morphologically from the one induced by other toxic substances like drugs, alcohol or pharmacological treatment.(3)

A French study in a limited territory estimated the incidence of toxic-induced liver disease to 14 per 100,000 inhabitants (4) and in the USA 15-30% of liver insufficiencies with fulminant development were correlated with occupational exposure to hepatotoxic agents.

The chemical agents present in some work environments are numerous and have an alarming growth rate. Table no.1 contains the main organic solvents with known hepatotoxic potential and the workplaces where these are encountered at.

1. Conditions for toxicity

The hepatotoxic effects are influenced by a series of factors:(5,6)

a. The aggressiveness of the toxic, defined by its capacity for selection/induction of enzymes implicated in biotransformation with the formation of bioactive metabolites and reactive oxygen species;
b. The affected population of cells;
c. The chronic or acute character of exposure;
d. The existence of differences in response between species;
e. Genetic factors implicating the isoform of the P_{450} cytochrome – $CYP2E_1$;
f. Hepatic blood flow;
g. The presence of some intrahepatic available sites, which enable the formation of bonds with the active metabolite of the xenobiotic;
h. The age of the person exposed to the risk;
i. Nutrition state;
j. Possible interactions between toxic and treatment/drug/alcohol;

Table no. 1. Organic solvents with known hepatotoxic potential and the workplaces they can be found in

Chemical compound	Type of lesion	Occupational exposure
alpha-naphthylisothio cyanate	Biliary ducts	Production of plastics and rubber
vinyl chloride	Hepatic fibrosis, angiosarcoma	Plastic products industry
chloroform	Necrosis of the hepatocyte	Diluent, nuclear spectroscopy, solvent
polychlorinated diphenyls	Subacute hepatic injury (hepatocytic necrosis)	Plasticizer, adhesives, insulating agents for transformers
dimethyl acetamide	Necrosis of the hepatocyte, steatosis	Solvent, manufacturing of resins, cosmetics
dimethylformamide	Acute hepatic injury (necrosis, steatosis)	Solvent, catalyst in chemical industry
dimethyl hydrazine	Hepatocytic necrosis, carcinogenic potential (2A)	Pesticide production and use
dimethyl nitrosamine	Hepatocellular carcinoma	Missile technology
dichlorhydrin	Central massive necrosis, steatosis	Glycerol synthesis
dioxin	Cutaneous porphyria+liver injury, cancer (?)	Pesticide, defoliant for incinerators
halothane	Hepatocellular necrosis, steatosis	anesthesiologists
methylenedianiline	Cholestasis, carcinogenesis (2B)	Mediator in the synthesis of isocyanates
naphthalene chlorinate	Hepatocytic necrosis, steatosis, regeneration	Impregnation of electrical equipment
2 nitropropane	Acute hepatic injury (hepatocytic necrosis), steatosis	Production of plastics, naval industry, food wrapings
carbon tetrachloride	Hepatocytic necrosis	Solvent, reagent in alcohol conversion
tetrachloroethane	Acute or subacute hepatic injury (hepatocytic necrosis). Carcinogenesis (2A).	Trichlorethylene and tetrachlorethylene production, refrigerant.
trichloroethane	Subacute hepatocytic necrosis, steatosis, cirrhosis	Degreaser with industrial use

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trichloroethylene	Centrolobular necrosis, steatosis, fibrosis	Degreasing agent, chemical cleaning
trinitrotoluene	Acute or subacute hepatic injury; macronodular cirrhosis, hepatocarcinoma	Ammunition manufacturing
xylene	hepatocytic necrosis	Painters, paint production, adhesives, plastics
toluene	Acute injury, hepatocytic necrosis, steatosis	Painters, paint production, adhesives, pharmaceutical and petrol industry

There are few toxic agents that determine anatomo-clinical effects in their original form, most of them becoming aggressive after biotransforming in the liver.

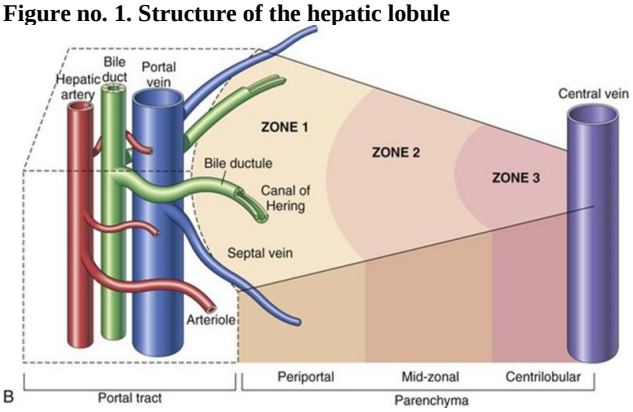
This process takes place in two phases: first, there are oxidation, reduction and hydrolysis reactions which transform the xenobiotic in intermediate products with reactive polar groups. It is at this level, during the second phase, that hydrosoluble compounds emerge from reactions like the conjugation of glicuronic acid, glutathione, sulphate a.o, which can be excreted especially through urine.(7)

The metabolic activation during the biotransformation process with the participation of the oxidases with mixt functions that belongs to the P450 cytochrome generates toxic metabolites and carcinogens. If these "critical" metabolites are not neutralized in enough manner or are formed in excessive quantities that exceeded the neutralising potential, then they will form adductions to the hepatocytic structures or additions to DNA with the help if their polar groups, thus initiating the mechanisms of toxicity and carcinogenesis.

2. The role of some structural topographical units in the mechanisms of toxicity

Satisfying hepatocytes with nutrients, but mainly with oxygen, depends upon the distance to the portal space. In the hepatic lobe, the hepatocytes are grouped along an axis between the portal space (where a branch of the hepatic artery, one of the portal veins and one biliary duct is found), and the centrolobular vein. Regarding the metabolic function, there one can distinguish 3 zones (figure no.1): first zone – periportal adjacent to the portal space, the third zone - adjacent to the centrolobular vein and the second zone - middle or intermediate, located between these two extreme zones.(8,9,10)

Figure no. 1. Structure of the hepatic lobule



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The higher partial O_2 pressure in the oxygenated blood coming from the hepatic artery favours the hepatocytes from the first zone, while the hepatocytes in the third zone, situated at a larger distance from the hepatic artery, have a lower O_2 offer at their disposal. The concentration of hepatotoxic lesions in the third zone is related to the O_2 gradient in the hepatic lobule, along this axis.(8) The third zone is the richest in P_{450} cytochrome, especially the ethanol inducible isoform CYP-$2E_1$, which occupies a very important role in metabolizing xenobiotics.(11)

Other structural elements with a potential to intervene in promoting liver toxicity are the Kupffer cells, a variety of macrophages, situated in the hepatic sinusoids, which can play a role of antigen presenting cells, seeing as they are a source of cytokines.(12) The activated Ito (stellate) cells, located in the Disse spaces, intervene in collagen synthesis, during the installation of fibrosis/cirrhosis.

3. Lesional determinations

Organic solvents develop hepatotoxic effects through diverse mechanisms depending on their chemical structure and properties. In table no. 2 we mention the most frequently implicated solvents and the way to determine their pathological effects.

Table no. 2. Categories of hepatotoxic agents and the type of induced pathologic effects

Category of agents	Mediation of effects	Examples
Agents with intrinsic (predictable) toxicity. Effects depending on dose.	**Direct hepatotoxicity** Direct injury of hepatocytes with necrosis and steatosis	carbon tetrachloride carbon tetrabromide tetrachloroethane chloroform trichloroethylene perchloroethylene
	Indirect hepatotoxicity Acting as antimetabolites, interfering in metabolically pathways necessary in maintaining the integrity of hepatocytes and the secretion of bile)	• **Hepatocytic cytotoxicity** (steatosis + necrosis): dimethyl nitrosamine ethanol • **Cholestasis** methylenedianiline dichloroethylene ethanol
Agents acting through idiosyncrasy. Effects independent from dose.	• Hypersensitivity mechanism • Production of toxic metabolites (cytochrome P_{450} polymorphism)	Phenytoin Halothane Isoniazid

The main types of injuries produced by organic solvents and in general by toxics with liver preference are: death of the hepatocytes, steatosis, cholestasis, cirrhosis and carcinogenesis.

3.1.The death of hepatocytes occurs in two ways: a.) necrosis by cellular ballooning due to the alteration of membrane permeability, dissolution of the nucleus and entrance of proinflammatory cells; b.) apoptosis or programmed death of the hepatocytes associated with the contraction of the cell, nuclear fragmentation and formation of the apoptotic body, with no inflammation.(13) The activation of apoptotic pathways is initiated via the TNF receptor or Fas with the launching of the caspase cascade. Most of the hepatotoxic agents cause the death of hepatocytes in the third zone, which can be explained by high levels of oxidases with mixt functions pertaining to the P_{450} cytochrome. At the same time, the first zone is somewhat protected by high levels of glutathione, the neutralizing effect of its free radicals being well-known. Hepatocytic necrosis induced by repeated exposures to hepatotoxic agents has likely an antibody-mediated immunological attack as trigger.(14)

3.2.Steatosis, defined as an increase of the fat content of the liver (over 5%), has generally a multiple determination: excessive supply of fatty acids towards the liver, increase in synthesis or esterification of fatty acids, decrease in their oxidation, interferences with the triglyceride cycle, decrease in synthesis of apoproteins needed for the formation of liver exportable lipoproteins. The synthesis occurs with high frequency because of exposure to trinitrotoluene, chlorate hydrocarbons, dimethylformamide.

In the third zone, the formation of the trichloromethyl radical (CCl_3^-) is followed by its covalent binding to the

hepatocytic components, causing the inhibition of lipoproteins' secretion and steatosis.(15)

3.3.Canalicular cholestasis consists of a retention of bile constituents inside the hepatocytes caused by the decrease in quantity of produced bile with the increase in blood concentration of biliary acids and bilirubin. Toxic metabolites can affect transport proteins at the level of the canalicular membrane and diminish, even interrupt the biliary flow.(13) The retention of biliary constituents prompts hepatocytic apoptosis by stimulating the translocation of Fas from the cytoplasm to the plasmatic membrane.(16) The injury to the intrahepatic biliary ducts is a pathologic condition described by Cullen and Reubner (17) under the name "cholangiodestructive cholestasis" and evolves with the increase in blood levels for biliary acids and cholesterol, similar to the phenomena in canalicular cholestasis. The industrial agent prototype with this kind of effect is methylenedianiline, used as a hardening agent for epoxi resins. Reference literature cites as a pointable example for methylenedianiline toxicity the "Epping Jaundice" epidemic that was recorded in the town of Epping in England, following the consumption of bread made from flour contaminated with this toxic: 84 people who consumed the contaminated product developed a severe hepatopathy on the background of intense biliary stasis, accompanied by light parenchymatous injuries.(18)

3.4.Cirrhosis assumes the existence of progressive necrosis injury of the hepatocytes, accompanied by the formation of regenerative nodules, fibrosis and architectural distortions of the liver structure ("toxic cirrhosis"). It can be a consequence of subacute hepatic necrosis, caused by trinitrotoluene, tetrachloroethane, polychlorinated diphenyls and naphthalene chlorinate. Prolonged and/or repeated exposure to low levels of carbon tetrachloride like the ones found in the dry cleaning process (3,5) can lead to cirrhosis.

3.5.Hepatic cancer can affect the hepatocytic line (carbon tetrachloride) or the sinusoidal endothelial cells, after exposure to arsenic, thorium dioxide or vinyl chloride. Among the first publications about the relationship between occupational exposure to vinyl chloride and angiosarcoma of the liver, we mention the paper published by the personnel of the Occupational Disease Clinic from Cluj-Napoca regarding the case of an exposed subject, employed in a vinyl chloride polymerization department.(19)

4. Pathogenetic mechanisms

The main mechanisms for inducing hepatotoxicity are: inflammation, cytochrome P_{450} malfunction, mitochondrial malfunction and oxidative stress.(1,2,5,6,8,10,11,15,16)

4.1.Inflammation. A key role in promoting inflammation is played by the Kupffer cells and the neutrophils. Through direct action, the toxic metabolite activates the Kupffer cells, which in turn will produce proinflammatory cytokines (IL-1, IL-6, TNF-α, a.o) that will stimulate the formation of an inflammatory infiltrate, lipogenesis, fibrogenesis and cholestasis.(13) Some cytokines adjust the genes that control the induction of apoptosis or stimulate hepatocytic proliferation.

Through a chemotactic signal of the Kupffer cells and the neutrophils activated by the xenobiotic, these transmigrate into the hepatic vascularisation, adhering to the hepatocyte. The phenomenon is followed by their degranulation with protease and high reactive oxygen species release that cause hepatocytic necrosis.(20) In experimental studies leukocytes' depletion diminished the hepatotoxic and cholestatic effects of alpha-naphthyl isothiocyanate ANIT (21) and pre-treatment with anti-inflammatory compounds reduced acute toxicity of ANIT and carbon tetrachloride. These observations confirm the role of inflammation in the hepatotoxicity of organic solvents.

4.2. Cytochrome P_{450} malfunction. Cytochrome P_{450}'s isoform CYP-$2E_1$ is the most abundant in the human liver and it operates mostly in the third zone of the hepatic lobule, intervening in the metabolism of numerous endo- and exogenous compounds. Genetic variability of enzymatic pathways, particularly at the cytochrome P_{450} level, as well as the 19 glutathione-glutathione peroxidase system variability, explains the existence of individual differences regarding the metabolism of xenobiotics and consequently the fluctuating responsiveness from one individual to another in similar conditions of exposure to the hazard. The decrease in cytochrome P_{450}, determined genetically or by inhibiting its synthesis, reduces the detoxification ability of solvents which is associated with the increase in fat liver content. For example, cobalt chloride and 3-amino-1,2,4-triazole inhibits the synthesis of Cytochrome P_{450}'s haem component. Preexposure to chemical agents stimulate the induction of mixt functions oxidases at microsomal level, thus favouring the biotransformation and toxicity of certain solvents. This explains the synergism observed in medical practice between ethanol abuse and carbon tetrachloride toxicity or the increase in metabolic clearance for m-xylene after pre-treatment with ethanol in experimental studies.

4.3. Mitochondrial malfunction. The compounds resulting during the bioactivation process act at mitochondrial level inhibiting beta-oxidation of fatty acids, which accumulate in the liver, a phenomenon that generates steatosis over time. These compounds also inhibit the sequentially of the Krebs cycle and the transfer of electrons along the respiratory chain, resulting in a decrease of the ATP level and the increase in reactive 19oxygen species which oxidate the lipid deposits.(22)

Oxidative stress is a disorder in the balance between prooxidants and antioxidants.(23) The main sources for free radicals are polymorphonuclear leukocytes and Kupffer cells that arrive in the outbreak of the reaction caused by the chemotactic message sent by the injured hepatocytes. In the formation of free radicals an important role is played by mixt functions oxidases, mainly the isoform CYP-$2E_1$. During the metabolic activation, the xenobiotics generate a large array of free radicals. For example, bioactivation of CCl_4 through CYP-$2E_1$ leads to the formation of trichloromethyl radical (CCl_3^-) which can bind to nucleic acids, proteins or lipids. The formation of DNA additions is an initiation pathway of carcinogenesis if the systems for excision/repair prove to be inefficient. The addition of these radicals to the proteins impedes the formation of lipoproteins contributing to development/worsening of the steatosis. The toxic effect of free radicals on the mechanism of lipoperoxidation also makes the membranes of inflammatory intervention cells fragile, leading to the release of other radicals, forming a vicious circle.

CONCLUSIONS

From the data mentioned above it can be stated that, the mechanisms that control hepatotoxicity are numerous, interdepended and present some unknown sides, suitable for further research.

This brief presentation aims to be a sensitizing message for systematic informing on occupational risk factors in subjects with acute or chronic liver injuries.

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