An Update on Adverse Outcome Pathways Leading to Liver Injury

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Abstract

Chemical-induced liver injury can be manifested in a number of ways, such as cholestasis, steatosis, fibrosis, and cancer. The mechanisms driving these toxicological processes have been well characterized and have been embedded in adverse outcome pathway frameworks in recent years. This article provides a concise overview of these constructs.

Keywords: AOP, cancer, cholestasis, fibrosis, hepatotoxicity, steatosis

Introduction

BECAUSE OF ITS UNIQUE FUNCTION and localization in the body, the liver is a primary target of toxicity induced by xenobiotics. Chemical-induced hepatotoxicity can be manifested in a number of prominent ways, such as cholestasis, steatosis, fibrosis, and cancer, and is a major reason for discontinuation of drug development or withdrawal of drugs from the market.^{1–3} It also is a concern for other sectors, including the cosmetics area.⁴ In this respect, it still remains challenging to detect and predict chemical-induced liver toxicity. Indeed, standard animal studies conducted during routine drug development usually pick up about half of all human hepatotoxic compounds, whereas human-based *in vitro* testing identifies up to 60% of the *in vivo* human hepatotoxic drugs.^{5,6} Adverse outcome pathways (AOPs) are promising tools in that regard, as they may help to predict chemical-induced liver toxicity in a more accurate and mechanistically anchored way. AOPs are conceptual constructs that portray existing knowledge concerning the linkage between a direct molecular initiating event (MIE) and an adverse outcome through a number of key events (KEs) at a biological level of organization relevant to risk assessment.^{7–9} In response to the increasing use of AOPs, the Organization for Economic Cooperation and Development together with the U.S. Environmental Protection Agency, the U.S. Army Engineer Research and Development Center, and the European Joint Research Center has introduced the AOP knowledge base (AOP-KB). The AOP-KB is composed of a number of modules, among which the AOP Wiki provides an open-source interface for rapid, widely accessible, and collaborative sharing of established AOPs and building new AOPs. The AOP Wiki contains several AOPs related to chemical-induced hepatotoxicity and liver pathology (Table 1), many of which are discussed hereafter.¹⁰

AOPs Leading to Liver Toxicity

Cholestasis

Cholestasis denotes any situation of impaired bile secretion with concomitant accumulation of potentially noxious cholephiles in the liver or in the systemic circulation. Only one AOP on this type of liver toxicity, in particular hepatocellular cholestasis, is currently available in the AOP-KB. The MIE in this AOP is the direct *cis*-inhibition of the bile salt export pump. As a result of this, toxic bile salts accumulate into hepatocytes or bile canaliculi. These bile salts trigger a direct deteriorative response and an adaptive response.¹ At the cellular level, the deteriorative response is accompanied by the formation of the mitochondrial permeability pore, which leads to mitochondrial impairment, inflammation, production of reactive oxygen species, and ultimately to the onset of cell death by both apoptotic and necrotic mechanisms.^{11,12} Because of the latter, cytosolic enzymes, including aminotransferases, start to leak from hepatocytes and cholangiocytes and become measurable in the serum.^{13,14} A hallmark of cholestasis at the cellular level includes the induction of an adaptive response, which is aimed at counteracting bile accumulation and thus cholestatic liver injury. Accordingly, a complex machinery of transcriptionally coordinated mechanisms involving nuclear receptors is activated by bile salts, which collectively decrease the uptake and increase the export of bile salts and bilirubin into and from hepatocytes, respectively. Simultaneously, detoxification of bile salts is enhanced, whereas their synthesis becomes downregulated.^{15–17} The increased effort of cholestatic hepatocytes to remove bilirubin causes bilirubinuria and hyperbilirubinemia. As a result, a yellowish pigmentation of the skin and the conjunctival membranes over the sclera becomes visible,

TABLE 1. ADVERSE OUTCOME PATHWAYS LEADING TO LIVER INJURY CURRENTLY (I.E., OCTOBER 2017) IN THE ADVERSE OUTCOME PATHWAY-KNOWLEDGE BASE⁹

<i>AOP-KB number</i>	<i>AOP title</i>	<i>OECD status</i>
27	Cholestatic liver injury induced by inhibition of the bile salt export pump	Under development
32	Inhibition of inducible nitric oxide synthase, hepatotoxicity, and regenerative proliferation leading to liver tumors	Under development
34	Liver X receptor activation leading to hepatic steatosis	Under development
36	Peroxisomal fatty acid beta-oxidation inhibition leading to steatosis	Under development
37	Peroxisome proliferator-activated receptor alpha-dependent liver cancer	Under development
38	Protein alkylation leading to liver fibrosis	TFHA/WNT endorsed
41	Sustained aryl hydrocarbon receptor activation leading to rodent liver tumors	EAGMST under review
57	Aryl hydrocarbon receptor activation leading to hepatic steatosis	Under development
58	Constitutive androstane receptor suppression leading to hepatic steatosis	Under development
59	Hepatocyte nuclear factor alpha suppression leading to hepatic steatosis	Under development
60	Pregnane X receptor activation leading to hepatic steatosis	Under development
61	Farnesoid X receptor activation leading to hepatic steatosis	Under development
62	Serine/threonine protein kinase 2 activation leading to hepatic steatosis	Under development
144	Lysosomal damage leading to liver inflammation	Under development
213	Inhibition of fatty acid beta oxidation leading to nonalcoholic hepatic steatosis	Under development
220	Chronic cytochrome P450 2E1 activation leading to liver cancer	Under development
232	Nuclear erythroid 2-related factor repression to steatosis	Under development
240	Deoxyribonucleic acid adducts leading to liver hemangiosarcoma	Under development

AOP-KB; adverse outcome pathway knowledge base; EAGMST, Expert Advisory Group on Molecular Screening and Toxicogenomics; OECD, Organization for Economic Cooperation and Development; TFHA, Task Force on Hazard Assessment; WNT, Working Group of the National Coordinators for the Test Guidelines Programme.

known as jaundice. Furthermore, the elevated presence of bile salts in the serum is thought to account for the typical skin itching in cholestasis patients.^{13,14,17}

Steatosis

Hepatic steatosis, also called fatty change, fatty degeneration, or adipose degeneration, is the process of abnormal retention of lipids, mainly triglycerides, within hepatocytes. It reflects the impairment of the normal processes of synthesis and elimination of triglycerides.¹ The AOP Wiki contains nine AOPs covering hepatic steatosis. Each of these AOPs considers a different MIE, including modulation of nuclear receptors (i.e., aryl hydrocarbon receptor, constitutive androstane receptor, farnesoid X receptor, liver X receptor, and pregnane X receptor), suppression of transcription factors (i.e., hepatocyte nuclear factor 4 alpha and nuclear erythroid 2-related factor), activation of serine/threonine kinase 2, and inhibition of peroxisomal fatty acid beta-oxidation. All these MIEs trigger an array of effects, such as enhanced transcription of genes encoding mediators of cholesterol and lipid metabolism, including carbohydrate response element binding protein, sterol response element binding protein 1c, fatty acid synthase, and stearoyl-coenzyme A desaturase 1. As a result, *de novo* synthesis of fatty acids is enhanced in the liver. At the same time, fatty acid translocase production is upregulated, which mediates increased hepatic influx of fatty acids from peripheral tissues. Consequently, triglycerides tend to accumulate in hepatocytes. At the organelle level, hepatocellular lipid accumulation may provoke cytoplasm displacement, nucleus distortion, mitochondrial toxicity, and endoplasmic reticulum stress. All together, these effects underlie the acquisition of the typical fatty liver cell phenotype, which, in turn, causes a clinically relevant increase in liver weight. Hepatic steatosis can develop further to nonalcoholic steatohepatitis, which is characterized by he-

patocellular injury and inflammation, and for which an AOP was recently included in the AOP-KB.¹⁰

Fibrosis

Liver fibrosis is a reversible wound healing response to either acute or chronic cellular injury that reflects a balance between liver repair and scar formation. A central event in liver fibrosis is the activation of hepatic stellate cells, which occurs in two phases, namely the initiation phase and the perpetuation phase. In the initiation phase, quiescent hepatic stellate cells become responsive to growth factors. This may be triggered by a variety of signals, including reactive oxygen species and apoptotic bodies originating from dying hepatocytes. In the perpetuation phase, the primed hepatic stellate cells undergo several changes related to proliferation, contractility, fibrogenesis, chemotaxis, extracellular matrix degradation, and retinoid loss, whereby they adopt a myofibroblast-like phenotype. Hepatic stellate cell activation may be counteracted in a resolution phase through apoptosis, senescence, or reversion to the quiescent phenotype. The most progressive form of fibrosis is cirrhosis, which, unlike fibrosis, is considered as an irreversible event.^{1,18–20} The AOP-KB contains one AOP on liver fibrosis in which protein alkylation is considered as the MIE. Different steps at the cellular and tissue level have been defined, including hepatocyte injury and cell death, activation of Kupffer cells, expression of transforming growth factor beta 1, activation of hepatic stellate cells, oxidative stress and chronic inflammation, collagen accumulation, and changes in hepatic extracellular matrix composition.¹⁰

Cancer

Chronic liver disease may burgeon into liver cancer, in particular hepatocellular carcinoma.¹ Today, four AOPs have been included in the AOP-KB related to liver cancer, all that

consider different MIEs, namely modulation of nuclear receptors (i.e., aryl hydrocarbon receptor and peroxisome proliferator-activated receptor α), chronic cytochrome P450 2E1 activation, and inhibition of inducible nitric oxide synthase. The latter two MIEs typically induce hepatotoxicity that, when maintained over extended periods of time, facilitates the onset of hepatocarcinogenesis. The four AOPs share a number of KEs related to disruption of the hepatic homeostatic balance in favor of cell growth, including decrease of apoptotic activity, increase in cell proliferation, hyperplasia in several liver cell types, and clonal expansion of preneoplastic foci cells. These events can be boosted by a number of modulating events, such as oxidative stress, activation of nuclear factor kappa beta, and inhibition of gap junctional intercellular communication. Recently, an AOP from DNA adducts formation to liver hemangiosarcoma was added to the AOP-KB.¹⁰

Perspectives

AOPs are to be considered as open and flexible constructs that should be continuously refined by feeding in relevant data. Such iterative refinement should ideally include the elaboration and quantification of the dynamic properties. Although quantification has now been pursued for a limited number of AOPs, the vast majority of them, including those related to liver injury, are still qualitative in nature.²¹ Future research should also be focused on creating AOP networks, which, unlike individual AOPs, are the actual functional tools for real-life applications, including serving as the backbone of integrated approaches to testing and assessment.^{8,9,21} An AOP network has already been proposed for liver steatosis.²² Furthermore, AOPs, *in casu* in the context of liver toxicity, may form the basis for setting up *in vitro* test batteries. This has also been recently demonstrated for liver steatosis.²³ Such efforts, and thus the advancement of the AOP field in general, should be strongly encouraged, as they open a plethora of perspectives for accurate and animal-free prediction of chemical-induced liver toxicity.

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