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Review Article

Pyrrolizidine alkaloids: An update on their metabolism and hepatotoxicity mechanism☆

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ABSTRACT

Pyrrolizidine alkaloids (PAs) are among the most hepatotoxic natural compounds that are widely distributed throughout the world. Most PAs are metabolically activated to trigger toxicity. Exposure to herbal medicine containing PAs and food supplements contaminated by PAs is considered to be one of the two main causes of hepatic sinusoidal obstruction syndrome (HSOS), which is a rare hepatic vascular disease with a high mortality rate. PAs-induced HSOS cases have been reported worldwide. However, there is no clinically effective therapy for PAs-induced HSOS, which is partially because the toxic mechanism is not fully understood. This review focuses on updating the information on the metabolism and the molecular mechanisms of PAs hepatotoxicity, including oxidative stress, apoptosis, and dysfunction of bile acid metabolism, and their interactions.

1. Introduction

Pyrrolizidine alkaloids (PAs) are alkaloids that typically contain either a saturated or a 1,2-unsaturated necine base unit. PAs are generally classified into four types based on the necine bases that are present, *i.e.,* platynecine, retronecine, heliotridine, and otonecine (Fig. 1). Except for the otonecine-type alkaloids, the other alkaloids can form the *N*-oxides, which are also commonly found in PAs-containing plants. In nature, about 660 PAs and PA *N*-oxides have been identified to date from over 6000 floriferous plants, which are mainly plants from the following four families: Compositae, Leguminosae, Orchidaceae, and Boraginaceae.[1]

PAs and their *N*-oxides are toxic to both humans and livestock through consumption of PAs-containing plants or food contaminated with PAs such as meat, milk, and honey.[1] Exposure to PAs has been regarded as one of the two major causes of hepatic sinusoidal obstruction syndrome (HSOS, previously called hepatic veno-occlusive disease), which is a rare hepatic vascular disease with a high mortality rate. The earliest case of PAs-induced HSOS was reported in 1920, and it was associated with ingestion of bread made from wheat that was contaminated with *Senecio* seeds in South Africa.[2] Because of the concern for human health and safety, numerous studies have been performed to illustrate the toxicity of PAs. Health and safety guidelines have been set by countries, such as Germany, Austria, and China, and international organizations, such as the World Health Organization. However, the number of PAs-induced poisoning reports is increasing. To date, over 8000 PAs-induced poisoning cases have been reported in many countries, including Russia, Indian, Afghanistan, Jamaican, South African, Australia, and the United States.[1] There is no clinically effective therapy for PAs-induced HSOS,[3] which is partially caused by the incomplete understanding of the toxic mechanism.

This review focuses on updating the information on the metabolism and the molecular mechanisms of PAs hepatotoxicity,

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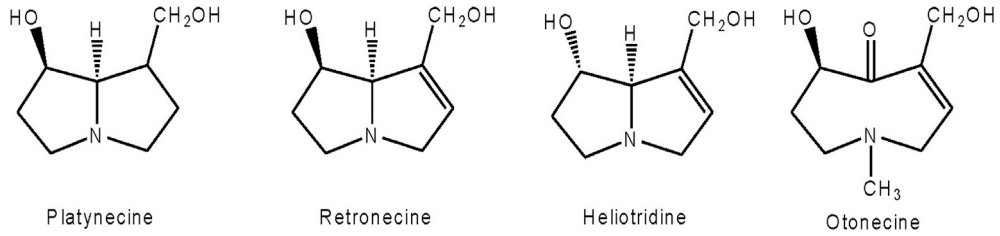

Fig. 1. Common necine bases in PAs. PAs are generally classified into four types based on the representative necine bases (platynecine, retronecine, heliotridine, and otonecine). The platynecine type of PAs contain a saturated necine base unit and are less toxic, while the other three types of PAs contain a 1,2-unsaturated necine base unit and are highly toxic. Abbreviation: PAs, pyrrolizidine alkaloids.

including oxidative stress, apoptosis, and dysfunction of bile acid metabolism, and their interactions.

2. PAs-induced HSOS

PAs can induce both chronic and acute liver injury. HSOS is characterized by edema, necrosis, detachment of endothelial cells in small sinusoidal hepatic and interlobular veins, intrahepatic congestion, portal hypertension, and liver dysfunction.[4–7] Without medical intervention and discontinuation of the toxicant exposure, HSOS can lead to more severe complications such as liver fibrosis, cirrhosis, necrosis, and ultimately death.[2] The etiologies of HSOS include cytoreductive therapy before hematopoietic stem cell transplantation (HSCT),[8–10] oxaliplatin-containing adjuvant chemotherapy,[11,12] consuming PAs-containing plants,[4,13] and using tacrolimus after liver transplantation.[14] In developed countries, HSOS usually occurs in patients who have received cytoreductive therapy before HSCT or oxaliplatin-containing chemotherapy for colorectal carcinoma.[9,15] In developing countries such as China, the primary cause of HSOS is the ingestion of PAs-containing herbs or dietary supplements.[4,16]

HSOS is characterized by toxic injury to the small hepatic vessels, particularly the sinusoidal endothelium in zone 3 of the liver acinus. Damaged sinusoids lead to sloughing and downstream occlusion of terminal hepatic venules.[17] A core pathogenic event of HSOS is toxic destruction of hepatic sinusoidal endothelial cells (HSECs).[18] The particular susceptibility of the HSECs is thought to be partially associated with increased activity of matrix metalloproteinases (MMPs), *e.g.*, MMP-9, which degrade the extracellular matrix and lead to the release of endothelial cells.[19–21] MMPs are normally suppressed by glutathione (GSH). As sinusoidal GSH levels rapidly decrease because of alkylation by dehydropyrrolizidine alkaloids (DHPAs) metabolites and the redox state of the sinusoid changes, MMP activity increases, and HSOS develops.[19,22] In addition, PAs can selectively decrease the GSH content in HSECs. Depletion of sinusoidal endothelial cell GSH has been a common mechanism that leads to HSOS (Fig. 2). Administration of GSH or its precursor N-acetyl-L-cysteine (NAC) was shown to confer protection against HSOS in monocrotaline (MCT)-treated rats. This can occur because GSH may conjugate with dehydromonocrotaline to form GSDHP, a compound of much lower toxicity that is released in high concentration into bile.[22,23] Biliary excretion of GSH and cysteinyl-glycine conjugates of MCT pyrrole support the role of thiol compounds in the detoxification of PAs.[24] However, this mechanism of PAs toxicity may not be universal because the embryotoxic effects of MCT cannot be prevented by feeding cysteine to pregnant rats.[25]

DeLeve *et al.*[26] found that a decrease in nitric oxide (NO) contributes to HSOS. NG-nitro-L-arginine methyl ester, an inhibitor of NO synthase, exacerbated MCT toxicity, whereas V-PYRRO/NO, a liver-selective NO donor prodrug, restored NO levels. Kumar *et al.*[27] revealed that although HSOS is considered to be a non-thrombotic vascular disease, sufficient evidence suggests that hemostatic derangement may be relevant to the occurrence of HSOS. The endothelial injury caused by PAs triggers the coagulation cascade and induces a hypercoagulable state.[28–30] In HSOS animal models, monocytes are recruited to the lobule and venous endothelium at an early stage.[31] Von Willebrand factor, thrombomodulin, and several cytokines, including tumor necrosis factor-alpha, interleukin-1 beta, endothelin-1, P-selection, and E-selection, are released by monocytes and/or endothelial cells in response to PAs toxicity.[32–35] Blood pyrrole-protein adducts (PPAs) are highly sensitive and specific for PAs-associated HSOS. The blood PPA concentration is related to the severity and clinical outcome of PAs-associated HSOS.[36] Damage to sinusoidal endothelial cells may prevent vascular endothelial growth factor receptor (VEGFR)-1 activation in these cells and the paracrine induction of hepatocyte growth through mitogenic mediators, such as hepatocyte growth factor that is produced by sinusoidal endothelial cells.[37]

In addition to upregulation of MMP expression, GSH depletion, NO reduction, coagulation pathways, and VEGF are involved in PAs-induced HSOS. Other evidence shows that bone marrow-derived progenitor cells can replace HSECs and central venous endothelial cells to repair complex damage, while MCT can inhibit endothelial progenitor cells in the bone marrow and circulation.[38] Thus, there are at least two key factors for PAs-induced HSOS, as follows: (i) toxic injury to sinusoidal/central venous endothelial cells; and (ii) toxic injury to bone marrow progenitors preventing the replacement of the injured endothelial cells in sinusoids and central veins.[17]

3. Metabolic activation of PAs

Metabolic activation is required for most PAs to exert toxicity.[39] It is well established that the PAs with an unsaturated necine base tend to be toxic. Generally, there are three principal metabolic pathways for PAs (Fig. 3). The most important of these pathways is oxidation via two steps, including hydroxylation of the necine base at the C3 or C8 position to form the corresponding 3- or 8-hydroxynecine derivatives followed by spontaneous dehydration to produce the corresponding dehydropyrrolizidine (pyrrolic ester) derivatives.[1,40] The second pathway is hydrolysis of the ester functional groups that are linked to the C7 and C9 positions to form the necine bases and the necic acids. The third pathway is *N*-oxidation of the necine bases to the corresponding PA *N*-oxides. Possessing a different necine base, otonecine-type PAs have two principal metabolic pathways: (i) formation of the corresponding pyrrolic esters through oxidative *N*-demethylation of the necine base followed by ring closure (via elimination of formaldehyde) and dehydration;[1,41] and (ii) hydrolysis of the ester functional groups to form the corresponding necine bases and acids. Recently, a new metabolic pathway, *i.e.*, *N*-glucuronidation of PAs via uridine

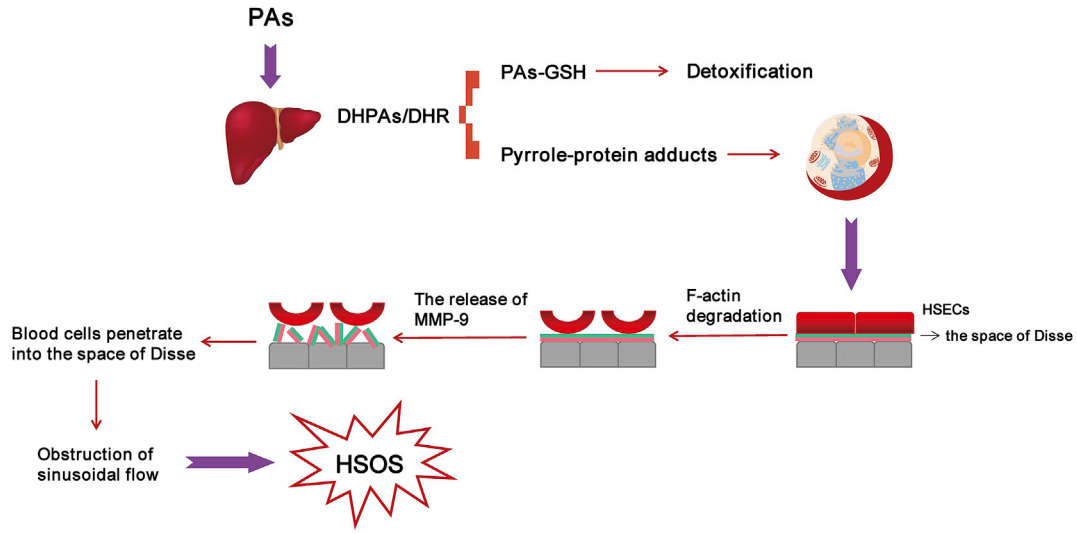

Fig. 2. Pathogenesis of PAs-induced HSOS. PAs are metabolically activated to generate the intermediate DHPAs and/or DHR, which can further bind with GSH to result in detoxification, or they can combine with protein to generate pyrrole–protein adducts to initiate HSOS. In HSECs, F-actin is depolymerized and MMP-9 is released to trigger extracellular matrix degradation. This increases the HSEC concentration and widens the gaps between HSECs and hepatocytes. The blood cells can then penetrate into the space of Disse, and the sinusoidal lining cells obstruct the sinusoidal flow, resulting in HSOS. Abbreviations: DHPAs, dehydropyrrolizidine alkaloids; DHR, dehydroretronecine; GSH, glutathione; HSECs, hepatic sinusoidal endothelial cells; HSOS, hepatic sinusoidal obstruction syndrome; MMP-9, matrix metalloproteinase-9; PAs, pyrrolizidine alkaloids.

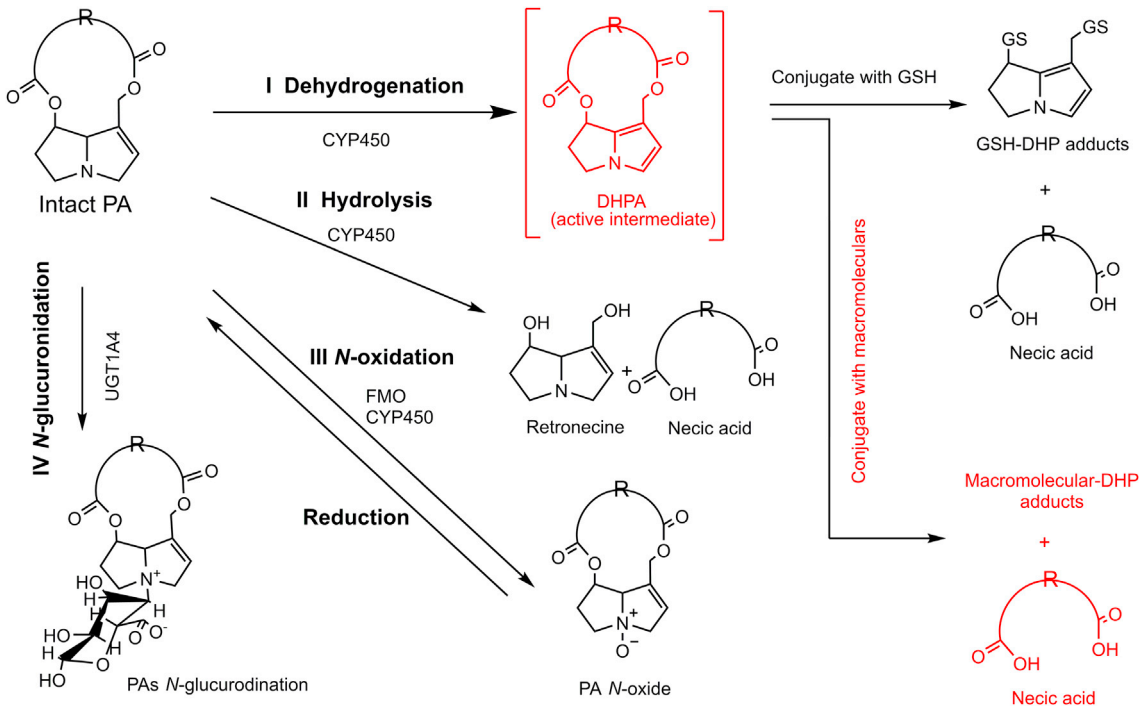

Fig. 3. Principal metabolism pathways of PAs. There are four major metabolic pathways for PAs, as follows: (**I**) dehydrogenation, (**II**) hydrolysis, (**III**) *N*-oxidation, and (**IV**) *N*-glucuronidation. Among them, dehydrogenation of PAs is recognized as the most important metabolic activation pathway because the DHPAs produced by dehydrogenation are highly electrophilic and can combine with essential cellular macromolecules such as proteins and nucleic acids to initiate toxicity. Abbreviations: CYP450, cytochrome P450; DHPA, dehydropyrrolizidine alkaloid; FMO, flavin-containing monooxygenase; GSH, glutathione; PA, pyrrolizidine alkaloid; UGT, uridine diphosphate glucuronosyltransferase.

diphosphate glucuronosyltransferase (UGT)1A4 (Fig. 3), was identified by He *et al.*[42,43]

PAs are metabolized predominantly by hepatocytes through cytochrome P450 (CYP450)-mediated transformation to *N*-oxides and conjugated dienic pyrroles.[39] These highly reactive, electrophilic pyrroles react with many biomolecules, such as GSH, which facilitate excretion. The pyrroles can also combine with essential cellular proteins and nucleic acids, forming adducts that may damage cell function and homeostasis. Hepatocytes and endothelial cells are most often affected.[44] For example, ATP synthase subunit beta (ATP5B) covalently bonds to the reactive pyrrolic metabolites of PAs to form the pyrrole-ATP5B adduct, which impairs mitochondrial function and significantly contributes to PAs-induced hepatotoxicity.[45] PA *N*-oxides may also be transformed to epoxides and toxic necines.[46–48] It has been found that the PA *N*-oxide metabolites, which are formed from metabolism of the

parent PAs *in vivo*, can be enzymatically reduced to the parent PAs in the gut and liver.[1,46,49,50] Consequently, the metabolism of PAs to PA *N*-oxides should be considered to be a potential, but minor, activation pathway.[46,49,51]

Metabolism of PAs to dehydropyrrolizidines is mainly catalyzed by CYP450, specifically both the CYP3A and CYP2B subfamilies.[52–57] CYP3A4 is the major enzyme that catalyzes the bioactivation and detoxication of senecionine in human liver.[58] CYP3A subfamilies are identified as the key enzymes in lasiocarpine metabolism.[59] CYP3A1 and CYP3A2 might play a significant role in the metabolic activation of clivorine in the rat.[60] Thus, these results support that the CYP3A subfamilies are the major metabolizing enzymes that are responsible for metabolic activation of toxic PAs.

Metabolism of PAs to the corresponding *N*-oxides is catalyzed by both CYP450 and flavin-containing monooxygenase (FMO).[53,58,61] For example, metabolism of senecionine to its *N*-oxide by microsomes of pig liver, lung, and kidney is mainly catalyzed by FMOs.[62,63] However, metabolism of senecionine by rat liver microsomes to its *N*-oxide is mainly catalyzed by CYP450 but not by the purified rabbit lung FMOs.[62]

In hepatocytes, CYP3A metabolizes about 50% of all common medications as well as estrogen, testosterone, and bile acids.[64] Thus, CYP3A inducers could increase the susceptibility of PAs-induced toxicity, while CYP3A inhibitors could prevent toxic outcomes because inhibitors yield fewer DHPAs.[1,50] Single-nucleotide polymorphisms (SNPs) in CYP3A5*3 and CYP3A5*6 were shown to result in the absence of CYP3A5 from tissues in some people, which contributes to the interindividual and interracial difference in CYP3A-dependent drug clearance.[65] Hepatic CYP3A4 expression varies 50-fold and the *in vivo* CYP3A4 enzymatic function (drug clearance) varies at least 20-fold among individuals.[66] Further research on SNPs in CYP3A4 may improve our understanding of variable individual susceptibility to PAs.

As the most important phase II drug-metabolizing enzymes, UGTs play an important role in the metabolism, excretion, and detoxification of many xenobiotics. However, the glucuronidation of intact PAs had not been investigated until Dr. He's work.[42] The major isozyme involved in *N*-glucuronidation of senecionine is UGT1A4, based on direct evidence that recombinant UGT1A4 exhibits predominant and exclusive activity on senecionine *N*-glucuronidation.[42] Additionally, *N*-glucuronidation of senecionine shows significant species differences. Rabbits, cattle, sheep, pigs, and humans show the significantly higher glucuronidation activity on senecionine compared with mice, rats, dogs, and guinea pigs. Other hepatotoxic PAs including MCT, adonifoline, and isoline also undergo *N*-glucuronidation in humans, rabbits, cattle, sheep, and pigs.[43] The species differences in *N*-glucuronidation of senecionine are consistent with the known toxicity reports. For example, rabbits show high activity on *N*-glucuronidation and are considered to be a resistant species to PAs toxicity.[1] Rats, mice, and dogs, which show nearly no *N*-glucuronidation activity, are susceptible to PA toxicity.[67] It has also been reported that the high non-CYP450 enzyme activity can significantly consume the intact PAs and prevent PAs from activating CYP450.[68]

4. Mechanism of PAs-induced hepatotoxicity

4.1. Oxidative stress and PAs-induced hepatotoxicity

Oxidative stress is an imbalance between reactive oxygen species (ROS) and the ability of the biological system to detoxify the reactive intermediates or to repair the damage caused by oxidative radicals.[69] Cellular redox balance is maintained by various enzymatic and nonenzymatic antioxidant systems. The disruption of this balance by exogenous substances will cause the overproduction of ROS, which cross-link cellular macromolecules such as DNA, RNA, proteins, or lipids and this leads to cellular damage.[70] ROSs are produced as a result of normal cellular metabolism within the mitochondria, CYP450 system, peroxisomes, and inflammatory cells.[71–73] PAs are reported to induce oxidative stress damage in various important organs, among which liver is the most sensitive organ.[74–76] Moreover, intracellular GSH and the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway play important roles in regulating PAs-induced hepatotoxicity.[74,77,78]

Cellular GSH, a ubiquitously distributed redox-active reducing sulfhydryl (−SH) tripeptide, is important for the maintenance of cellular redox state via directly scavenging radical species or participating in the reactions that are catalyzed by antioxidant enzymes such as glutathione peroxidase (GPx) and glutathione-S-transferase (GST).[79–81] Glutamate-cysteine ligase (GCL) and glutathione reductase (GR) are two critical enzymes for the regulation of cellular GSH amounts,[82] which can protect cells against oxidative damage.[19,74] Senecionine and other PAs, such as adonifoline, MCT, and isoline, dose- and time-dependently deplete cellular GSH levels in L-02 cells and increase the level of oxidized GSH.[19,83] NAC, the precursor to GSH, lowered the susceptibility of PAs-induced hepatotoxicity,[19,74] while a GSH synthesis inhibitor increases the susceptibility to PAs-induced hepatotoxicity.[74] In addition, PA clivorine decreases the activity of superoxide dismutase in rat hepatocytes, which is consistent with the effects of clivorine on GSH-related antioxidant enzymes such as GPx, GST, and GR.[84] Another study showed that the cellular total antioxidant capacity and the GST activity increased in clivorine-treated L-02 cells, while clivorine decreased GR activity in cells.[85] Similar results are also found in rodents that were treated with PAs.[86,87] Therefore, intracellular GSH and its related enzymes such as GPx, GST, GCL, and GR constitute the crucial antioxidant system for counteracting ROS production during PAs-induced oxidative stress.[80,88] The imbalance of the antioxidant system leads to PAs-induced hepatotoxicity.

The Kelch-like ECH-associated protein1 (Keap1)-Nrf2 pathway is key for cellular stressors such as ROS and reactive electrophilic metabolites. Cellular stressors ultimately result in the dissociation of the Keap1-Nrf2 complex and activation of Nrf2 as a transcription factor.[89] Nrf2 is a transcription factor with a high sensitivity to oxidative stress that regulates the expression of many antioxidants and detoxification genes by binding to antioxidant response elements (AREs).[90–92] Liquiritigenin and liquiritin are reported to potentially interact with the Nrf2 binding site in the Keap1 protein.[78] They alleviated MCT-induced HSOS by dissociation of Nrf2 with Keap1, which led to Nrf2 nuclear translocation.[78] Another study showed that (−)-epicatechin increased Nrf2 nuclear translocation in rats that were treated with MCT and enhanced the expression of Nrf2 downstream antioxidant genes including glutamate cysteine ligase catalytic and modifier subunits (GCLC and GCLM) and NAD(P)H: quinine oxidoreductase 1 (NQO1), which protect against MCT-induced injury.[93] Similarly, several other natural compounds, including (+)-catechin hydrate, chlorogenic acid, quercetin, and baicalein, also attenuated MCT-induced HSOS by via inducing Nrf2-mediated transcriptional activation or nuclear translocation (Fig. 4).[94–96]

4.2. Apoptosis and PAs-induced hepatotoxicity

Apoptosis, which is one of the main forms of cell death, is a mode of regulated cell death that is required for morphogenesis, development, and homeostasis of multicellular organisms.[97] The two most important groups of proteins involved in apoptosis, *i.e.*, cysteine-dependent aspartate-specific proteases (caspases) and the B-cell lymphoma 2 (Bcl-2) family of proteins, participate in all apoptotic cell death pathways.[98,99] Caspases function as both the

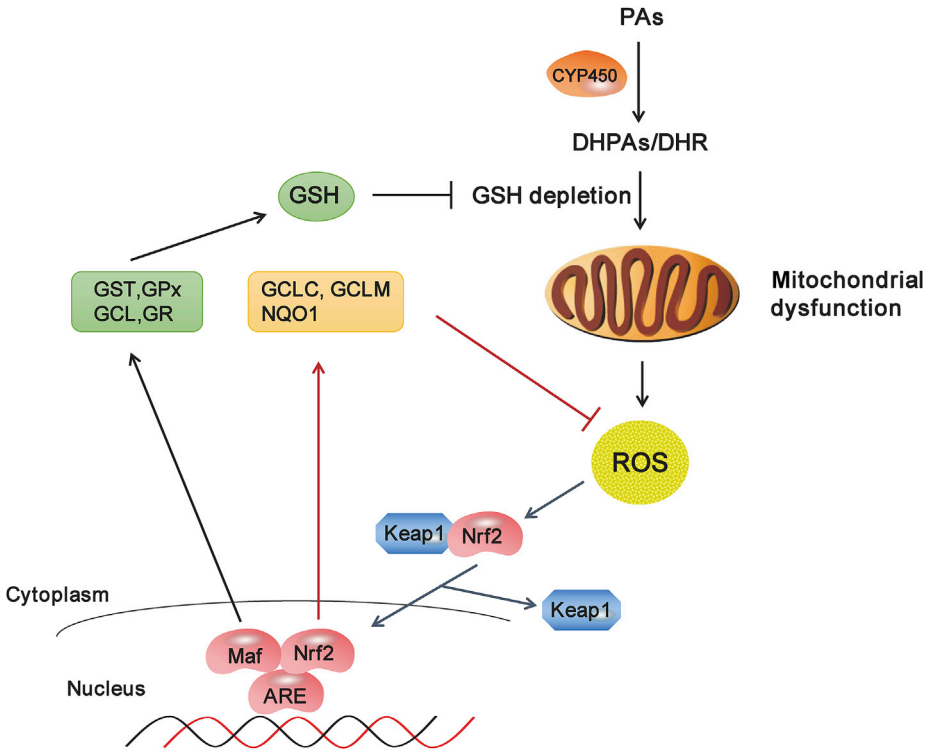

Fig. 4. PAs induce liver injury through oxidative stress. PAs are first metabolized by CYP450 and generate DHPAs and/or DHR, which in turn deplete hepatic GSH and cause mitochondrial dysfunction, resulting in overproduction of ROS. ROS, in turn, leads to activation of signaling events including Nrf2-mediated activation of genes containing ARE. Nrf2 nuclear translocation and the Nrf2-mediated antioxidant defense system contribute to preventing excessive ROS levels from accumulating at the cellular and tissue levels. Abbreviations: ARE, antioxidant response element; CYP450, cytochrome P450; DHPAs, dehydropyrrolizidine alkaloids; DHR, dehydroretronecine; GCL, glutamate-cysteine ligase; GCLC, glutamate cysteine ligase catalytic; GCLM, glutamate cysteine ligase modifier; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GST, glutathione-S-transferase; Keap1, Kelch-like ECH-associated protein1; NQO1, NAD(P)H: quinine oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor 2; PAs, pyrrolizidine alkaloids; ROS, reactive oxygen species.

initiators (*e.g.*, caspase-2, -8, -9, -10) and executors (*e.g.*, caspase-3, -6, -7) of apoptotic cell death.[97] Bcl-2 family members function as regulators of almost all known forms of apoptosis and comprise the following three subfamilies: the anti-apoptotic subfamily (*e.g.*, Bcl-xl, Bcl-2), the multi-domain pro-apoptotic subfamily (*e.g.*, Bax, Bak), and the pro-apoptotic BH3-only subfamily (*e.g.*, Bim, Bad).[100] Previous studies on both of *in vivo* and *in vitro* models demonstrated that PAs can induce hepatotoxicity via activation of apoptosis.[101–103] Both the intrinsic pathway (mitochondrial pathway) and the extrinsic pathway (death receptor pathway) are implicated in PAs-induced apoptosis.

Clivorine induces apoptosis by degradation of anti-apoptotic Bcl-xl and increases the release of cytochrome *c* from mitochondria to cytosol, leading to the activation of caspase-9/caspase-3 signaling cascade.[104] Moreover, senecionine induces dynamin-related protein 1 (Drp1)-mediated mitochondrial fragmentation both *in vivo* and *in vitro*, suggesting a key role for mitochondria fission and fusion in PAs-induced toxicity. Mitochondrial division inhibitor-1 (Mdivi), a selective Drp1 inhibitor, attenuates senecionine-induced mitochondrial fragmentation/depolarization, mitochondrial release of cytochrome *c*, and apoptosis.[105] All these studies illustrate the possible involvement of the mitochondrial intrinsic pathway in PAs-induced apoptosis in hepatocytes.

Another study demonstrated that metabolically activated lasiocarpine induces Fas-mediated apoptosis in human liver HepG2 cells, indicating a possible involvement of the extrinsic apoptotic pathway.[106] Overexpression of Bcl-x or the related protein Bcl-2 in transgenic mice confers a survival advantage against Fas-induced hepatic apoptosis and hepatic ischemia reperfusion, respectively.[107,108] Moreover, PAs-induced apoptosis in the human liver HepaRG cells was related to the participation of the caspase-8 and caspase-9, which is likely occurs through a mechanism involving both the death receptor pathway (extrinsic) and the mitochondrial pathway (intrinsic) (Fig. 5).[109]

4.3. Dysfunction of bile acid metabolism and PAs-induced hepatotoxicity

Bile acids are the major endogenous metabolites of cholesterol.[110] They can interact with the farnesoid X receptor (FXR) and G-protein-coupled bile acid receptor (TGR5) and stimulate several other nuclear hormone and G-protein-coupled receptors.[111,112] Bile acids have, therefore, emerged as versatile signaling molecules, regulating their own homoeostasis as well as adjusting cholesterol and lipid homoeostasis.[113] In the entire bile acid circulation process, numerous enzymes mediate the rate-limiting steps in the biosynthesis pathway, including cholesterol 7α-hydroxylase (CYP7A1). When bile acid levels are high, the CYP7A1 enzyme activity will be repressed by a nuclear receptor cascade involving FXR, which suppresses the synthesis of bile acids and maintains the balance in the bile acid pool.[114] The maintenance of bile acid concentrations below certain levels is important to avoid liver toxicity because bile acids are also detergents that may damage cellular membranes and trigger hepatocellular death.[115] In the liver, this is achieved partially through the effect of bile acids on their intracellular receptor FXR, a bile acid-activated transcriptional regulator that controls the expression of genes that are involved in the synthesis, conjugation, uptake, and secretion of these molecules, among other metabolic regulatory pathways.[116–118] Bile acids have been evaluated as biomarkers in various liver diseases such as nonalcoholic

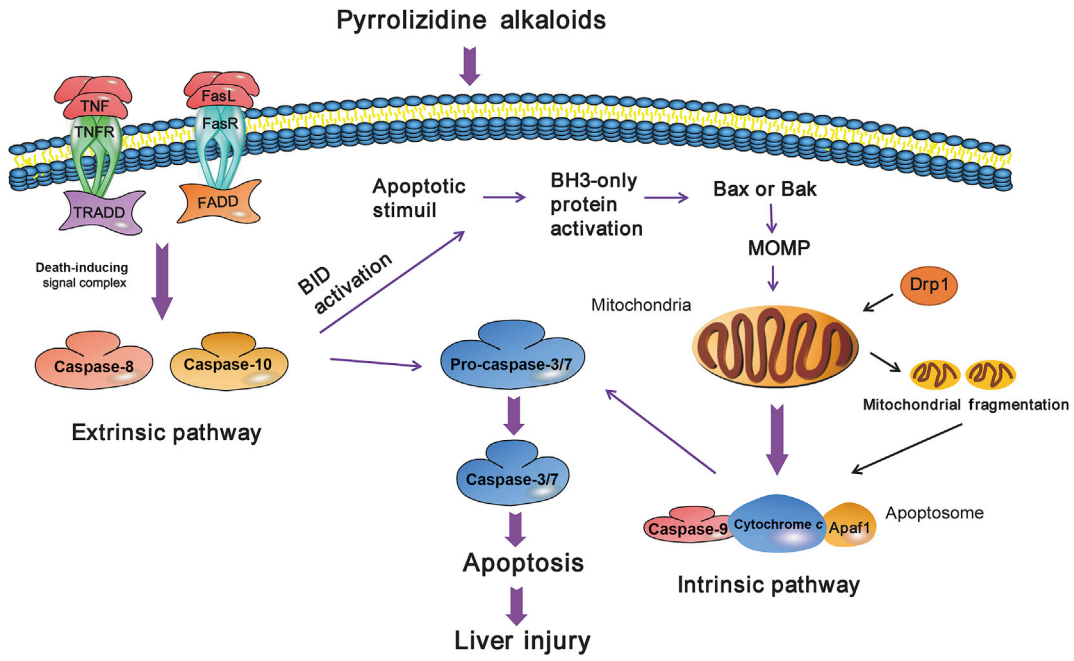

Fig. 5. PAs induce extrinsic (death receptor) and intrinsic (mitochondrial) apoptotic pathways. The first step in the initiation of the PAs-induced extrinsic pathway is binding of the death ligands to their respective receptors on the plasma membrane, as follows: TNF binds with TNFR and FasL binds with FasR. This is followed by the binding of TRADD and/or FADD to the intracellular domains of the death receptors. These reactions result in the formation of a death-inducing signaling complex, which promotes the activation of caspase-8 and -10. Once they are in the active state, they either activate executioner caspase-3 and -7, which results in apoptosis, or converge onto the intrinsic pathway via BID activation (mitochondrial amplification loop). However, the intrinsic pathway is initiated in response to apoptotic stimuli, which activates BH3-only pro-apoptotic proteins from the Bcl2 family. Bax and/or Bak are consequently activated and induce MOMP. Cytochrome *c* is released from the mitochondria. Cytochrome *c*, together with apoptotic protease Apaf-1 and caspase-9, form the apoptosome. Caspase-9 is activated in the apoptosome and subsequently activates caspase-3 and -7, which eventually leads to apoptosis and liver injury. PAs also induce Drp1 mitochondrial translocation to trigger mitochondrial fragmentation, increased mitochondrial cytochrome *c* release, and apoptosis. Abbreviations: Apaf-1, apoptotic protease activating factor 1; Drp1, dynamin-related protein 1; FADD, Fas-associated death domain; FasL, Fas ligand; FasR, Fas receptor; MOMP, mitochondrial outer membrane permeabilization; PAs, pyrrolizidine alkaloids; TNF, tumor necrosis factor; TNFR, TNF receptor; TRADD, TNF receptor-associated death domain.

steatohepatitis, hepatocellular carcinoma, and cirrhosis.[119–121] In addition, bile acids are also important biomarkers for the evaluation of hepatotoxicity that is caused by exposure to PAs-containing herbs.[122,123]

Decreased release of bile acids in PAs-perfused livers and an increased serum level of bile acids were observed in PAs-treated animals.[124–127] Previous studies have suggested that increased serum bile acids could be a sensitive index of hepatic function caused by PAs.[125,126] Recent studies by Xiong *et al.*[128–130] indicate that compromised bile acids homeostasis plays an important role in PAs-induced hepatotoxicity. Through a combination of metabolomics and genomics studies, a significantly altered bile acids profile is revealed in rats that were exposed to senecionine, which is related to the changes in ATP-binding cassette transporters, primary bile acid biosynthesis, and bile excretion.[128] Further study showed that the detoxification process by conjugation of free bile acids to glycine and taurine via bile acid-CoA amino acid *N*-acetyltransferase is hindered, which enhances hepatic bile acid accumulation and results in liver injury.[128]

A similar phenomenon is observed in herbal medicines containing PAs, such as *Senecio vulgaris* and *Gynura japonica*,[129,130] both of which have caused severe HSOS. The serum profile of bile acids, along with hepatic transcriptional factors, is significantly changed after PAs-containing herb treatment. The free and conjugated bile acids increase, and the organism shows adaptive modulation of bile acids to prevent bile acid overload by suppressing bile acid synthesis, limiting bile acid reabsorption, and reducing bile acid accumulation in hepatocytes by increasing basolateral excretion.[129] Exposure to *G. japonica* causes liver injury through direct injury to hepatocytes and cholestasis, and through impaired bile acid homeostasis.[130] However, the bile acid profile that is impaired by PAs is different from that caused by other chemicals such as α-naphthylisothiocyanate, carbon tetrachloride, acetaminophen, and allyl alcohol.[130] Bile acid homeostasis may, therefore, serve as a powerful key point for classifying different phenotypes of liver injury. Bile acids, especially those that are hydrophobic, are known to be cytotoxic to liver cells. Dysfunction of bile acid receptors such as FXR can increase bile acid synthesis and hinder bile acid secretion from the liver, and thus, result in hepatic bile acid overload that induces liver injury. Senecionine reduces the hepatic expression of FXR and its small heterodimer partner,[128] suggesting that it may inhibit FXR activation. However, CYP7A1, which should be activated under conditions of FXR dysfunction, also decreases at the same time.[128] Therefore, it is difficult to clarify whether PAs-induced bile acid dysregulation is a direct reason for the hepatotoxicity or if it is a secondary effect that is caused by PAs-induced hepatotoxicity. Further research, especially the time- and dose-relationship between PA toxicity and bile acid signaling pathway, should be performed to clarify the underlying mechanism of PAs-induced liver injury.

5. Conclusion

PAs may be the most common poison that affects livestock, wildlife, and humans around the world. Although several health and safety guidelines have been set for the use of PAs-containing herbs and preparations, PAs-induced toxin cases are still reported worldwide and there are no effective clinical therapies. Thus, more effort should be continuously exerted to provide a better understanding of these biotransformations and the toxic mechanism. It is also important to determine the sources of human exposure, as

well as the human health risk that is posed by these compounds, and to reduce exposure to these compounds.

Authors' contributions

L. Yang and A. Xiong defined the research theme and designed the study; J. Xu and A. Xiong wrote and revised the manuscript; W. Wang and X. Yang summarized part of literatures; L. Yang and Z. Wang supervised the study. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. Fu PP, Xia Q, Lin G, Chou MW. Pyrrolizidine alkaloids-genotoxicity, metabolism enzymes, metabolic activation, and mechanisms. *Drug Metab Rev*. 2004;36:1–55.
2. Willmot F, Robertson G. Senecio disease, or cirrhosis of the liver, due to senecio poisoning. *Lancet*. 1920;196:848–849.
3. Cooperative Group for Hepatic and Gall Diseases, Chinese Society of Gastroenterology, Chinese Medical Association. Expert consensus on diagnosis and treatment of pyrrolidine alkaloids-related sinusoidal obstruction syndrome. *J Clin Hepatol*. 2017;33:1627–1637.
4. Wang JY, Gao H. Tusanqi and hepatic sinusoidal obstruction syndrome. *J Dig Dis*. 2014;15:105–107.
5. Gao H, Li N, Wang JY, Zhang SC, Lin G. Definitive diagnosis of hepatic sinusoidal obstruction syndrome induced by pyrrolizidine alkaloids. *J Dig Dis*. 2012;13:33–39.
6. Lin G, Wang JY, Li N, et al. Hepatic sinusoidal obstruction syndrome associated with consumption of Gynura segetum. *J Hepatol*. 2011;54:666–673.
7. Xu Z, Zhuge YZ, Xu T. Raise the recognition of hepatic veno-occlusive disease induced by chrysanthemum-like groundsel. *Chin J Gastroenterol*. 2009;14:577–579.
8. Plessier A, Rautou PE, Valla DC. Management of hepatic vascular diseases. *J Hepatol*. 2012;56:S25–S38.
9. Dignan FL, Wynn RF, Hadzic N, et al. BCSH/BSBMT guideline: diagnosis and management of veno-occlusive disease (sinusoidal obstruction syndrome) following haematopoietic stem cell transplantation. *Br J Haematol*. 2013;163:444–457.
10. Yakushijin K, Atsuta Y, Doki N, et al. Sinusoidal obstruction syndrome after allogeneic hematopoietic stem cell transplantation: incidence, risk factors and outcomes. *Bone Marrow Transplant*. 2016;51:403–409.
11. Liu F, Cao X, Ye J, Pan X, Kan X, Song Y. Oxaliplatin-induced hepatic sinusoidal obstruction syndrome in a patient with gastric cancer: a case report. *Mol Clin Oncol*. 2018;8:453–456.
12. Han NY, Park BJ, Kim MJ, Sung DJ, Cho SB. Hepatic parenchymal heterogeneity on contrast-enhanced CT scans following oxaliplatin-based chemotherapy: natural history and association with clinical evidence of sinusoidal obstruction syndrome. *Radiology*. 2015;276:766–774.
13. Zhuge YZ, Wang Y, Zhang F, et al. Clinical characteristics and treatment of pyrrolizidine alkaloid-related hepatic vein occlusive disease. *Liver Int*. 2018;38:1867–1874.
14. Shen T, Feng XW, Geng L, Zheng SS. Reversible sinusoidal obstruction syndrome associated with tacrolimus following liver transplantation. *World J Gastroenterol*. 2015;21:6422–6426.
15. Morine Y, Shimada M, Utsunomiya T. Evaluation and management of hepatic injury induced by oxaliplatin-based chemotherapy in patients with hepatic resection for colorectal liver metastasis. *Hepatol Res*. 2014;44:59–69.
16. Wang X, Qi X, Guo X. Tusanqi-related sinusoidal obstruction syndrome in China: a systematic review of the literatures. *Medicine (Baltimore)*. 2015;94:e942.
17. Valla DC, Cazals-Hatem D. Sinusoidal obstruction syndrome. *Clin Res Hepatol Gastroenterol*. 2016;40:378–385.
18. Fan CQ, Crawford JM. Sinusoidal obstruction syndrome (hepatic veno-occlusive disease). *J Clin Exp Hepatol*. 2014;4:332–346.
19. DeLeve LD, Shulman HM, McDonald GB. Toxic injury to hepatic sinusoids: sinusoidal obstruction syndrome (veno-occlusive disease). *Semin Liver Dis*. 2002;22:27–42.
20. DeLeve LD, Ito Y, Bethea NW, McCuskey MK, Wang X, McCuskey RS. Embolization by sinusoidal lining cells obstructs the microcirculation in rat sinusoidal obstruction syndrome. *Am J Physiol Gastrointest Liver Physiol*. 2003;284:G1045–G1052.
21. Nolan JP, Scheig RL, Klatskin G. Delayed hepatitis and cirrhosis in weanling rats following a single small dose of the senecio alkaloid, lasiocarpine. *Am J Pathol*. 1966;49:129–151.
22. Wang X, Kanel GC, DeLeve LD. Support of sinusoidal endothelial cell glutathione prevents hepatic veno-occlusive disease in the rat. *Hepatology*. 2000;31:428–434.
23. Chen Z, Huo JR. Hepatic veno-occlusive disease associated with toxicity of pyrrolizidine alkaloids in herbal preparations. *Neth J Med*. 2010;68:252–260.
24. Lamé MW, Jones AD, Morin D, Segall HJ, Wilson DW. Biliary excretion of pyrrolic metabolites of [14C] monocrotaline in the rat. *Drug Metab Dispos*. 1995;23:422–429.
25. Soto-Blanco B, Medeiros RM, Guerra JL, Górniak SL. Lack of protective action of cysteine against the fetotoxic effect of monocrotaline. *Food Chem Toxicol*. 2001;39:635–639.
26. DeLeve LD, Wang X, Kanel GC, et al. Decreased hepatic nitric oxide production contributes to the development of rat sinusoidal obstruction syndrome. *Hepatology*. 2003;38:900–908.
27. Kumar S, DeLeve LD, Kamath PS, Tefferi A. Hepatic veno-occlusive disease (sinusoidal obstruction syndrome) after hematopoietic stem cell transplantation. *Mayo Clin Proc*. 2003;78:589–598.
28. Baron F, Deprez M, Beguin Y. The veno-occlusive disease of the liver. *Haematologica*. 1997;82:718–725.
29. Peres E, Kintzel P, Dansey R, et al. Early intervention with antithrombin III therapy to prevent progression of hepatic veno-occlusive disease. *Blood Coagul Fibrinolysis*. 2008;19:203–207.
30. Lee JH, Lee KH, Kim S, et al. Relevance of proteins C and S, antithrombin III, von Willebrand factor, and factor VIII for the development of hepatic veno-occlusive disease in patients undergoing allogeneic bone marrow transplantation: a prospective study. *Bone Marrow Transplant*. 1998;22:883–888.
31. DeLeve LD, McCuskey RS, Wang X, et al. Characterization of a reproducible rat model of hepatic veno-occlusive disease. *Hepatology*. 1999;29:1779–1791.
32. Wadleigh M, Ho V, Momtaz P, Richardson P. Hepatic veno-occlusive disease: pathogenesis, diagnosis and treatment. *Curr Opin Hematol*. 2003;10:451–462.
33. Coppell JA, Brown SA, Perry DJ. Veno-occlusive disease: cytokines, genetics, and haemostasis. *Blood Rev*. 2003;17:63–70.
34. Collins PW, Gutteridge CN, O'Driscoll A, et al. von Willebrand factor as a marker of endothelial cell activation following BMT. *Bone Marrow Transplant*. 1992;10:499–506.
35. Castenskiold EC, Kelsey SM, Collins PW, et al. Functional hyperactivity of monocytes after bone marrow transplantation: possible relevance for the development of post-transplant complications or relapse. *Bone Marrow Transplant*. 1995;15:879–884.
36. Gao H, Ruan JQ, Chen J, et al. Blood pyrrole-protein adducts as a diagnostic and prognostic index in pyrrolizidine alkaloid-hepatic sinusoidal obstruction syndrome. *Drug Des Dev Ther*. 2015;9:4861–4868.
37. LeCouter J, Moritz DR, Li B, et al. Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. *Science*. 2003;299:890–893.
38. Harb R, Xie G, Lutzko C, et al. Bone marrow progenitor cells repair rat hepatic sinusoidal endothelial cells after liver injury. *Gastroenterology*. 2009;137:704–712.
39. Mattocks AR. Toxicity of pyrrolizidine alkaloids. *Nature*. 1968;217:723–728.
40. Fu PP, Chou MW, Xia Q, et al. Genotoxic pyrrolizidine alkaloids and pyrrolizidine alkaloid N-oxides: mechanisms leading to DNA adduct formation and tumorigenicity. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev*. 2008;19:353–385.
41. Lin G, Cui YY, Hawes EM. Characterization of rat liver microsomal metabolites of clivorine, an hepatotoxic otonecine-type pyrrolizidine alkaloid. *Drug Metab Dispos*. 2000;28:1475–1483.
42. He YQ, Liu Y, Zhang BF, et al. Identification of the UDP-glucuronosyltransferase isozyme involved in senecionine glucuronidation in human liver microsomes. *Drug Metab Dispos*. 2010;38:626–634.
43. He YQ, Yang L, Liu HX, et al. Glucuronidation, a new metabolic pathway for pyrrolizidine alkaloids. *Chem Res Toxicol*. 2010;23:591–599.
44. Stegelmeier BL, Colegate SM, Brown AW. Dehydropyrrolizidine alkaloid toxicity, cytotoxicity, and carcinogenicity. *Toxins*. 2016;8:E356.
45. Lu Y, Ma J, Song Z, Ye Y, Fu PP, Lin G. The role of formation of pyrrole-ATP synthase subunit beta adduct in pyrrolizidine alkaloid-induced hepatotoxicity. *Arch Toxicol*. 2018;92:3403–3414.
46. Robins DJ. *Chemistry and Toxicology of Pyrrolizidine Alkaloids*. London: Academic Press; 1986:2698–2699.
47. Couet CE, Crews C, Hanley AB. Analysis, separation, and bioassay of pyrrolizidine alkaloids from comfrey (Symphytum officinale). *Nat Toxins*. 1996;4:163–167.

48. Stickel F, Seitz HK. The efficacy and safety of comfrey. *Public Health Nutr*. 2000;3:501–508.
49. Chou MW, Wang YP, Yan J, et al. Riddelliine *N*-oxide is a phytochemical and mammalian metabolite with genotoxic activity that is comparable to the parent pyrrolizidine alkaloid riddelliine. *Toxicol Lett*. 2003;145:239–247.
50. Wang YP, Yan J, Fu PP, Chou MW. Human liver microsomal reduction of pyrrolizidine alkaloid *N*-oxides to form the corresponding carcinogenic parent alkaloid. *Toxicol Lett*. 2005;155:411–420.
51. Xia Q, Zhao Y, Von Tungeln LS, et al. Pyrrolizidine alkaloid-derived DNA adducts as a common biological biomarker of pyrrolizidine alkaloid-induced tumorigenicity. *Chem Res Toxicol*. 2013;26:1384–1396.
52. Dueker SR, Lamé MW, Morin D, Wilson DW, Segall HJ. Guinea pig and rat hepatic microsomal metabolism of monocrotaline. *Drug Metab Dispos*. 1992;20:275–280.
53. Chung WG, Miranda CL, Buhler DR. A cytochrome P4502B form is the major bioactivation enzyme for the pyrrolizidine alkaloid senecionine in Guinea pig. *Xenobiotica*. 1995;25:929–939.
54. Kasahara Y, Kiyatake K, Tatsumi K, et al. Bioactivation of monocrotaline by P-450 3A in rat liver. *J Cardiovasc Pharmacol*. 1997;30:124–129.
55. Huan JY, Miranda CL, Buhler DR, Cheeke PR. The roles of CYP3A and CYP2B isoforms in hepatic bioactivation and detoxification of the pyrrolizidine alkaloid senecionine in sheep and hamsters. *Toxicol Appl Pharmacol*. 1998;151:229–235.
56. Xia Q, Chou MW, Kadlubar FF, Chan PC, Fu PP. Human liver microsomal metabolism and DNA adduct formation of the tumorigenic pyrrolizidine alkaloid, riddelliine. *Chem Res Toxicol*. 2003;16:66–73.
57. Ruan J, Yang M, Fu P, Ye Y, Lin G. Metabolic activation of pyrrolizidine alkaloids: insights into the structural and enzymatic basis. *Chem Res Toxicol*. 2014;27:1030–1039.
58. Miranda CL, Reed RL, Guengerich FP, Buhler DR. Role of cytochrome P450IIIA4 in the metabolism of the pyrrolizidine alkaloid senecionine in human liver. *Carcinogenesis*. 1991;12:515–519.
59. Ebmeyer J, Braeuning A, Glatt H, These A, Hessel-Pras S, Lampen A. Human CYP3A4-mediated toxification of the pyrrolizidine alkaloid lasiocarpine. *Food Chem Toxicol*. 2019;130:79–88.
60. Lin G, Cui YY, Liu XQ. Gender differences in microsomal metabolic activation of hepatotoxic clivorine in rat. *Chem Res Toxicol*. 2003;16:768–774.
61. Ramsdell HS, Buhler DR. Microsomal metabolism of pyrrolizidine alkaloids: *N*-oxidation of seneciphylline and senecionine. *Toxicol Lett*. 1987;37:241–249.
62. Williams DE, Reed RL, Kedzierski B, Ziegler DM, Buhler DR. The role of flavin-containing monooxygenase in the *N*-oxidation of the pyrrolizidine alkaloid senecionine. *Drug Metab Dispos*. 1989;17:380–386.
63. Miranda CL, Chung W, Reed RE, et al. Flavin-containing monooxygenase: a major detoxifying enzyme for the pyrrolizidine alkaloid senecionine in Guinea pig tissues. *Biochem Biophys Res Commun*. 1991;178:546–552.
64. Gura T. Genetics. Can SNPs deliver on susceptibility genes? *Science*. 2001;293:593–595.
65. Kuehl P, Zhang J, Lin Y, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet*. 2001;27:383–391.
66. Eichelbaum M, Burk O. CYP3A genetics in drug metabolism. *Nat Med*. 2001;7:285–287.
67. Huan JY, Miranda CL, Buhler DR, Cheeke PR. Species differences in the hepatic microsomal enzyme metabolism of the pyrrolizidine alkaloids. *Toxicol Lett*. 1998;99:127–137.
68. Lin G, Tang J, Liu XQ, Jiang Y, Zheng J. Deacetylclivorine: a gender-selective metabolite of clivorine formed in female Sprague-Dawley rat liver microsomes. *Drug Metab Dispos*. 2007;35:607–613.
69. Betteridge DJ. What is oxidative stress? *Metabolism*. 2000;49:3–8.
70. Sies H. Biochemistry of oxidative stress. *Angew Chem Int Ed*. 1986;23:1798.
71. Inoue M, Sato EF, Nishikawa M, et al. Mitochondrial generation of reactive oxygen species and its role in aerobic life. *Curr Med Chem*. 2003;10:2495–2505.
72. Dröse S, Brandt U. Molecular mechanisms of superoxide production by the mitochondrial respiratory chain. *Adv Exp Med Biol*. 2012;748:145–169.
73. Zangar RC, Davydov DR, Verma S. Mechanisms that regulate production of reactive oxygen species by cytochrome P450. *Toxicol Appl Pharmacol*. 2004;199:316–331.
74. Ji L, Liu T, Chen Y, Wang Z. Protective mechanisms of N-acetyl-cysteine against pyrrolizidine alkaloid clivorine-induced hepatotoxicity. *J Cell Biochem*. 2009;108:424–432.
75. Liu TY, Chen Y, Wang ZY, Ji LL, Wang ZT. Pyrrolizidine alkaloid isoline-induced oxidative injury in various mouse tissues. *Exp Toxicol Pathol*. 2010;62:251–257.
76. Amin KA, Hashem KS, Al-muzafar HM, Taha EM. Oxidative hepatotoxicity effects of monocrotaline and its amelioration by lipoic acid, S-adenosyl methionine and vitamin E. *J Complement Integr Med*. 2014;11:35–41.
77. Chen Y, Ji L, Wang H, Wang Z. Intracellular glutathione plays important roles in pyrrolizidine alkaloids-induced growth inhibition on hepatocytes. *Environ Toxicol Pharmacol*. 2009;28:357–362.
78. Huang Z, Sheng Y, Chen M, Hao Z, Hu F, Ji L. Liquiritigenin and liquiritin alleviated MCT-induced HSOS by activating Nrf2 antioxidative defense system. *Toxicol Appl Pharmacol*. 2018;355:18–27.
79. Walker RM, Racz WJ, McElligott TF. Acetaminophen-induced hepatotoxicity in mice. *Lab Investig*. 1980;42:181–189.
80. DeLeve LD, Kaplowitz N. Glutathione metabolism and its role in hepatotoxicity. *Pharmacol Ther*. 1991;52:287–305.
81. Lei XG. In vivo antioxidant role of glutathione peroxidase: evidence from knockout mice. *Methods Enzymol*. 2002;347:213–225.
82. Griffith OW. Biologic and pharmacological regulation of mammalian glutathione synthesis. *Free Radic Biol Med*. 1999;27:922–935.
83. Nuringtyas TR, Verpoorte R, Klinkhamer PG, van Oers MM, Leiss KA. Toxicity of pyrrolizidine alkaloids to Spodoptera exigua using insect cell lines and injection bioassays. *J Chem Ecol*. 2014;40:609–616.
84. Ji L, Liu T, Wang Z. Pyrrolizidine alkaloid clivorine induced oxidative injury on primary cultured rat hepatocytes. *Hum Exp Toxicol*. 2010;29:303–309.
85. Liang QN, Liu TY, Ji LL, Min Y, Xia YY. Pyrrolizidine alkaloid clivorine-induced oxidative stress injury in human normal liver L-02 cells. *Drug Discov Ther*. 2009;3:247–251.
86. Amin KA, Hassan MS, Awad el-ST, Hashem KS. The protective effects of cerium oxide nanoparticles against hepatic oxidative damage induced by monocrotaline. *Int J Nanomed*. 2011;6:143–149.
87. Liang QN, Sheng YC, Jiang P, et al. The difference of glutathione antioxidant system in newly weaned and young mice liver and its involvement in isoline-induced hepatotoxicity. *Arch Toxicol*. 2011;85:1267–1279.
88. McConnachie LA, Mohar I, Hudson FN, et al. Glutamate cysteine ligase modifier subunit deficiency and gender as determinants of acetaminophen-induced hepatotoxicity in mice. *Toxicol Sci*. 2007;99:628–636.
89. Giudice A, Montella M. Activation of the Nrf2-ARE signaling pathway: a promising strategy in cancer prevention. *Bioessays*. 2006;28:169–181.
90. Kensler TW, Wakabayashi N, Biswal S. Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway. *Annu Rev Pharmacol Toxicol*. 2007;47:89–116.
91. Niture SK, Khatri R, Jaiswal AK. Regulation of Nrf2-an update. *Free Radic Biol Med*. 2014;66:36–44.
92. Chen B, Lu Y, Chen Y, Cheng J. The role of Nrf2 in oxidative stress-induced endothelial injuries. *J Endocrinol*. 2015;225:R83–R99.
93. Huang Z, Jing X, Sheng Y, et al. (-)-Epicatechin attenuates hepatic sinusoidal obstruction syndrome by inhibiting liver oxidative and inflammatory injury. *Redox Biol*. 2019;22:101117.
94. Zheng Z, Shi L, Sheng Y, Zhang J, Lu B, Ji L. Chlorogenic acid suppresses monocrotaline-induced sinusoidal obstruction syndrome: the potential contribution of NFκB, Egr1, Nrf2, MAPKs and PI3K signals. *Environ Toxicol Pharmacol*. 2016;46:80–89.
95. Jing X, Zhang J, Huang Z, Sheng Y, Ji L. The involvement of Nrf2 antioxidant signaling pathway in the protection of monocrotaline-induced hepatic sinusoidal obstruction syndrome in rats by (+)-catechin hydrate. *Free Radic Res*. 2018;52:402–414.
96. Zhang J, Sheng Y, Shi L, et al. Quercetin and baicalein suppress monocrotaline-induced hepatic sinusoidal obstruction syndrome in rats. *Eur J Pharmacol*. 2017;795:160–168.
97. Kopeina GS, Prokhorova EA, Lavrik IN, et al. Alterations in the nucleocytoplasmic transport in apoptosis: caspases lead the way. *Cell Prolif*. 2018;51:e12467.
98. Mcllwain DR, Berger T, Mak TW. Caspase functions in cell death and disease. *Cold Spring Harb Perspect Biol*. 2013;5:a008656.
99. Taylor RC, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Biol*. 2008;9:231–241.
100. Cory S, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. *Nat Rev Cancer*. 2002;2:647–656.
101. Copple BL, Rondelli CM, Maddox JF, Hoglen NC, Ganey PE, Roth RA. Modes of cell death in rat liver after monocrotaline exposure. *Toxicol Sci*. 2004;77:172–182.
102. Luckert C, Hessel S, Lenze D, Lampen A. Disturbance of gene expression in primary human hepatocytes by hepatotoxic pyrrolizidine alkaloids: a whole genome transcriptome analysis. *Toxicol In Vitro*. 2015;29:1669–1682.
103. Steenkamp V, Stewart MJ, van der Merwe S, Zuckerman M, Crowther NJ. The effect of Senecio latifolius a plant used as a South African traditional medicine, on a human hepatoma cell line. *J Ethnopharmacol*. 2001;78:51–58.
104. Ji L, Chen Y, Liu T, Wang Z. Involvement of Bcl-xL degradation and mitochondrial-mediated apoptotic pathway in pyrrolizidine alkaloids-induced apoptosis in hepatocytes. *Toxicol Appl Pharmacol*. 2008;231:393–400.
105. Yang X, Wang H, Ni HM, et al. Inhibition of Drp1 protects against senecionine-induced mitochondria-mediated apoptosis in primary hepatocytes and in mice. *Redox Biol*. 2017;12:264–273.
106. Ebmeyer J, Franz L, Lim R, et al. Sensitization of human liver cells towards Fas-mediated apoptosis by the metabolically activated pyrrolizidine alkaloid lasiocarpine. *Mol Nutr Food Res*. 2019;63:1801206.
107. Mitchell C, Mallet VO, Guidotti JE, Goulenok C, Kahn A, Gilgenkrantz H. Liver repopulation by BCL-x(L) transgenic hepatocytes. *Am J Pathol*. 2002;160:31–35.
108. Selzner M, Rüdiger HA, Selzner N, Thomas DW, Sindram D, Clavien PA. Transgenic mice overexpressing human Bcl-2 are resistant to hepatic ischemia and reperfusion. *J Hepatol*. 2002;36:218–225.
109. Waizenegger J, Braeuning A, Templin M, Lampen A, Hessel-Pras S. Structure-dependent induction of apoptosis by hepatotoxic pyrrolizidine alkaloids in the human hepatoma cell line HepaRG: single versus repeated exposure. *Food Chem Toxicol*. 2018;114:215–226.
110. Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem*. 2003;72:137–174.

111. Schaap FG, Trauner M, Jansen PL. Bile acid receptors as targets for drug development. *Nat Rev Gastroenterol Hepatol*. 2014;11:55–67.
112. Hofmann AF, Hagey LR. Bile acids: chemistry, pathochemistry, biology, pathobiology, and therapeutics. *Cell Mol Life Sci*. 2008;65:2461–2483.
113. Arab JP, Karpen SJ, Dawson PA, Arrese M, Trauner M. Bile acids and nonalcoholic fatty liver disease: molecular insights and therapeutic perspectives. *Hepatology*. 2017;65:350–362.
114. Chiang JY. Bile acids: regulation of synthesis. *J Lipid Res*. 2009;50:1955–1966.
115. Perez MJ, Briz O. Bile-acid-induced cell injury and protection. *World J Gastroenterol*. 2009;15:1677–1689.
116. Wang H, Chen J, Hollister K, Sowers LC, Forman BM. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol Cell*. 1999;3:543–553.
117. Matsubara T, Li F, Gonzalez FJ. FXR signaling in the enterohepatic system. *Mol Cell Endocrinol*. 2013;368:17–29.
118. Preidis GA, Kim KH, Moore DD. Nutrient-sensing nuclear receptors PPARα and FXR control liver energy balance. *J Clin Investig*. 2017;127:1193–1201.
119. Ferslew BC, Xie G, Johnston CK, et al. Altered bile acid metabolome in patients with nonalcoholic steatohepatitis. *Dig Dis Sci*. 2015;60:3318–3328.
120. Chen T, Xie G, Wang X, et al. Serum and urine metabolite profiling reveals potential biomarkers of human hepatocellular carcinoma. *Mol Cell Proteom*. 2011;10, 004945. M110.
121. Mannes GA, Thieme C, Stellaard F, Wang T, Sauerbruch T, Paumgartner G. Prognostic significance of serum bile acids in cirrhosis. *Hepatology*. 1986;6:50–53.
122. Stegelmeier BL, Gardner DR, James LF, Molyneux RJ. Pyrrole detection and the pathologic progression of Cynoglossum officinale (houndstongue) poisoning in horses. *J Vet Diagn Investig*. 1996;8:81–90.
123. Luckert C, Hessel S, Lenze D, Lampen A. Disturbance of gene expression in primary human hepatocytes by hepatotoxic pyrrolizidine alkaloids: a whole genome transcriptome analysis. *Toxicol In Vitro*. 2015;29:1669–1682.
124. Lessard P, Wilson WD, Olander HJ, Rogers QR, Mendel VE. Clinicopathologic study of horses surviving pyrrolizidine alkaloid (Senecio vulgaris) toxicosis. *Am J Vet Res*. 1986;47:1776–1780.
125. Mendel VE, Witt MR, Gitchell BS, et al. Pyrrolizidine alkaloid-induced liver disease in horses: an early diagnosis. *Am J Vet Res*. 1988;49:572–578.
126. Sutherland RJ, Deol HS, Hood PJ. Changes in plasma bile acids, plasma amino acids, and hepatic enzyme pools as indices of functional impairment in liver-damaged sheep. *Vet Clin Pathol*. 1992;21:51–56.
127. Yan CC, Huxtable RJ. Relationship between glutathione concentration and metabolism of the pyrrolizidine alkaloid, monocrotaline, in the isolated, perfused liver. *Toxicol Appl Pharmacol*. 1995;130:132–139.
128. Xiong A, Yang F, Fang L, et al. Metabolomic and genomic evidence for compromised bile acid homeostasis by senecionine, a hepatotoxic pyrrolizidine alkaloid. *Chem Res Toxicol*. 2014;27:775–786.
129. Xiong A, Fang L, Yang X, et al. An application of target profiling analyses in the hepatotoxicity assessment of herbal medicines: comparative characteristic fingerprint and bile acid profiling of Senecio vulgaris L. and Senecio scandens Buch.-Ham. *Anal Bioanal Chem*. 2014;406:7715–7727.
130. Xiong A, Shao Y, Fang L, et al. Comparative analysis of toxic components in different medicinal parts of Gynura japonica and its toxicity assessment on mice. *Phytomedicine*. 2019;54:77–88.