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Cytotoxicity of pyrrolizidine alkaloid in human hepatic parenchymal and sinusoidal endothelial cells: Firm evidence for the reactive metabolites mediated pyrrolizidine alkaloid-induced hepatotoxicity

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ABSTRACT

Pyrrolizidine alkaloids (PAs) widely distribute in plants and can cause hepatic sinusoidal obstruction syndrome (HSOS), which typically presents as a primary sinusoidal endothelial cell damage. It is well-recognized that after ingestion, PAs undergo hepatic cytochromes P450 (CYPs)-mediated metabolic activation to generate dehydropyrrolizidine alkaloids (DHPAs), which are hydrolyzed to dehydroretronecine (DHR). DHPAs and DHR are reactive metabolites having same core pyrrole moiety, and can bind proteins to form pyrrole-protein adducts, which are believed as the primary cause for PA-induced HSOS. However, to date, the direct evidences supporting the toxicity of DHPAs and DHR in the liver, in particular in the sinusoidal endothelial cells, are lacking. Using human hepatic sinusoidal endothelial cells (HSEC) and HepG2 (representing hepatic parenchymal cells), cells that lack CYPs activity, this study determined the direct cytotoxicity of dehydromonocrotaline, a representative DHPA, and DHR, but no cytotoxicity of the intact PA (monocrotaline) in both cell lines, confirming that reactive metabolites mediate PA intoxication. Comparing with HepG2, HSEC had significantly lower basal glutathione (GSH) level, and was significantly more susceptible to the reactive metabolites with severer GSH depletion and pyrrole-protein adducts formation. The toxic potency of two reactive metabolites was also compared. DHPA was more reactive than DHR, leading to severer toxicity. In conclusion, our results unambiguously provided the first direct evidence for the critical role of DHPA and DHR in the reactive metabolites-mediated PA-induced hepatotoxicity, which occurs predominantly in HSEC due to severe GSH depletion and the significant formation of pyrrole-protein adducts in HSEC.

1. Introduction

Pyrrolizidine alkaloids (PAs) are probably one of the most significant groups of natural phytotoxins. More than 660 PAs and PA *N*-oxides have been identified in over 6000 plants distributed in many geographical regions worldwide, and about half of these phytochemicals have been reported to be hepatotoxic in human and livestock [1,2]. Humans exposed to toxic PAs through the consumption of PA-containing plants used as herbal remedies, teas and salads, and PA-contaminated dietary products, including wheat, milk, honey, pollen, eggs, and meat [3–11]. Very high amounts of PAs have been found in dietary products including teas [6] and retail honey [5,7], and consumption of PA-contaminated grains has caused large and recurring episodes of acute hepatotoxicity in a numbers of countries [12,13]. Since it was first found in 1920, more than 10,000 PA-poisoning cases have been documented worldwide with the most cases caused by the exposure to PA-contaminated foodstuffs [8,12–14]. As such, PA contamination has caused a severe public health problem. It is also possible that many

Abbreviations: CYP, cytochrome P450; DABA, 4-(dimethylamino)benzaldehyde; DHPA, dehydropyrrolizidine alkaloid; DHR, dehydroretronecine; ECM, endothelial cell medium; FBS, fetal bovine serum; GSH, glutathione; HSEC, human hepatic sinusoidal endothelial cells; HSOS, hepatic sinusoidal obstruction syndrome; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NADPH, nicotinamide adenine dinucleotide phosphate; PA, pyrrolizidine alkaloid; UHPLC-MS, ultrahigh high pressure liquid chromatography-mass spectrometry.

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more PA intoxication cases may not be documented due to the lack of a well-established record system and the unavailability of confirmative diagnostic methods [15].

PAs are hepatotoxic and mainly cause hepatic sinusoidal obstruction syndrome (HSOS) typically with hepatomegaly, hyperbilirubinaemia, and ascites [16]. PA intoxication is generally believed to be due to the metabolic activation [11,15]. As illustrated in Fig. 1, the two types of toxic PAs, namely retronecine-type and otonecine-type, undergo oxidation and oxidative *N*-demethylation mediated by cytochromes P450 (CYPs) to produce the corresponding metabolites, dehydropyrrolizidine alkaloids (DHPAs), which contain an identical necine base (core pyrrole moiety) regardless of their corresponding parent PAs. DHPAs are chemically reactive electrophilic metabolites with extremely short half-lives [17]. Once formed, DHPAs rapidly interact with water and cellular constituents, and thus have never been directly detected in any *in vivo* or *in vitro* biological systems. On the other hand, the formation of DHPA has been confirmed by unequivocal identification of the metabolites generated from further biotransformation of DHPAs [18–20]. It is well established that DHPAs bind with cellular macromolecules like proteins to form 2,3-dihydro-1H-pyrrolizine-protein (pyrrole–protein) adducts, which was firstly suggested by Mattocks in 1968 [21] and has been well recognized nowadays as the initiation of PA-induced hepatotoxicity [20,22,23]. Recently our group has developed a highly sensitive UHPLC-MS method to detect and quantify pyrrole-protein adducts in the liver and blood of rats treated with PAs and also in the blood of patients who consumed PA-containing medicinal herbs [8,15,24]. In addition, DHPAs can also bind with glutathione (GSH) to form pyrrole-GSH conjugates [19], which have been found in various *in vitro* metabolic preparations and also in the bile of the PA-treated animals, revealing that glutathione conjugation is a principal detoxification pathway in metabolism-mediated PA intoxication [18–20,25].

Hydrolysis of DHPAs generates dehydroretronecine (DHR), which is also a reactive electrophilic metabolite. A previous *in vitro* metabolic study had demonstrated DHR as the hydrolyzed metabolite of DHAPs [22]. Similar to DHPAs, DHR can bind with GSH or proteins to generate pyrrole-GSH conjugates or pyrrole-protein adducts respectively leading to detoxification or hepatotoxicity [23,26]. However, DHR is less chemically reactive but has higher hydrophilicity than DHPAs, and is readily excreted in bile and urine of the PA-treated animals [10,11,18,26]. Therefore, comparing with DHPAs, DHR is considered to be a less reactive and less toxic metabolite of PAs [11,22]. The metabolic biotransformation between the pyrrole-protein adduction and the detoxification pathways, such as GSH conjugation, appears to be crucial in determining the hepatotoxicity of PAs [10,11]. Although the metabolic pathways of PAs and the bioactivation of PAs to DHPAs and DHR have been delineated for a long time, to date, the direct toxicity resulted in the intervention of proteins with the reactive metabolites, DHPAs and DHR, which is a critical step in the PA-induced hepatotoxicity, has not been firmly confirmed.

Furthermore, unlike most of the drug-induced liver injury, PA-induced hepatotoxicity, particularly HSOS, usually presents as a primary sinusoidal damage followed by the parenchymal cell dysfunction, which indicates the primary vascular nature of the disease [16]. Several animal models have been established to demonstrate that PAs specifically damage the liver sinusoids [16,27–29]. Histological features of PA-treated rats showed extensive loss of sinusoidal cells, entrance of red blood cells into the space of Disse, and disruption of the hepatocyte plasma membrane [16,27,29]. Based on previous animal studies of PA-induced HSOS, PA-induced liver damage occurs primarily and predominantly in hepatic sinusoidal endothelial cells, although it also occurs in parenchymal cells [16,27,29]. Based on the metabolic activation-mediated PA intoxication, there are at least two critical factors hypothesized to be highly responsible for determining which cell type is more vulnerable to PA intoxication: 1) the difference in GSH level, which is strongly related to the detoxification ability and intracellular redox balance, and 2) the difference in pyrrole-protein

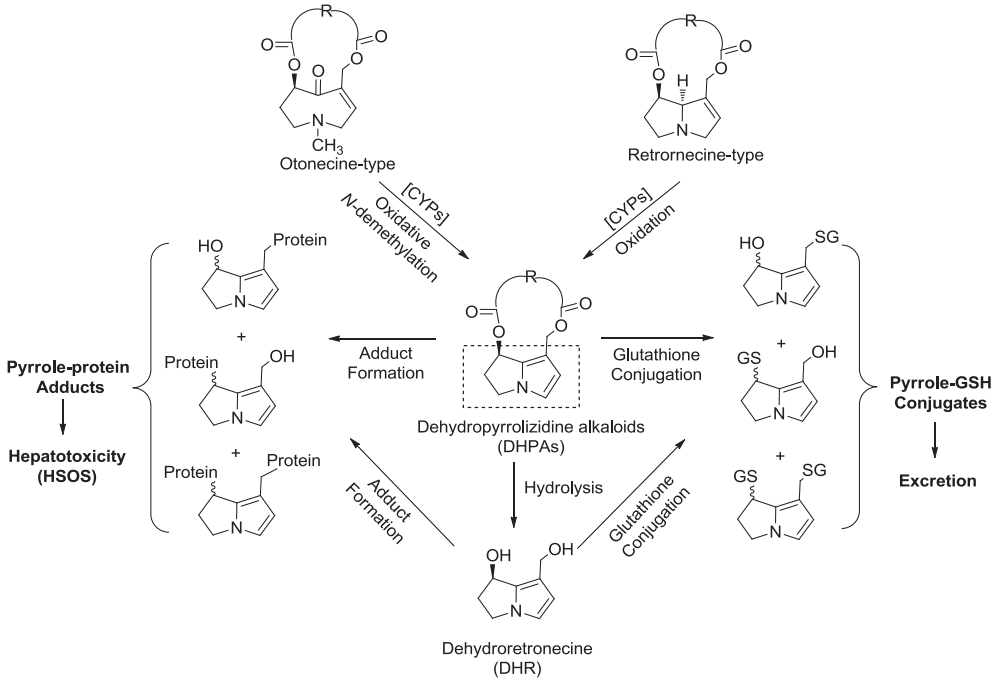

Fig. 1. Proposed hepatic metabolic activation of retronecine-type and otonecine-type PAs to form dehyropyrrolizidine alkaloids (DHPAs), which are hydrolyzed into dehydroretronecine (DHR). DHPAs and DHR further interacts with glutathione or proteins to generate pyrrole-GSH conjugates or pyrrole-protein adducts, respectively. Core pyrrole moiety is indicated in the dashed box.