

Pyrrolizidine alkaloids in human diet

Arungundrum S. Prakash^{a,*}, Tamara N. Pereira^a, Paul E.B. Reilly^b,
Alan A. Seawright^a

^a National Research Centre for Environmental Toxicology, 39, Kessels Road, Coopers Plains, QLD 4108, Australia

^b Department of Biochemistry, The University of Queensland, St. Lucia, QLD 4072, Australia

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Abstract

Pyrrolizidine alkaloids are the leading plant toxins associated with disease in humans and animals. Upon ingestion, metabolic activation in liver converts the parent compounds into highly reactive electrophiles capable of reacting with cellular macromolecules forming adducts which may initiate acute or chronic toxicity. The pyrrolizidine alkaloids present a serious health risk to human populations that may be exposed to them through contamination of foodstuffs or when plants containing them are consumed as medicinal herbs. Some pyrrolizidine alkaloids (PA) adducts are persistent in animal tissue and the metabolites may be re-released and cause damage long after the initial period of ingestion. PAs are also known to act as teratogens and abortifacients. Chronic ingestion of plants containing PAs has also led to cancer in experimental animals and metabolites of several PAs have been shown to be mutagenic in the *Salmonella typhimurium*/mammalian microsome system. However, no clinical association has yet been found between human cancer and exposure to PAs. Based on the extensive reports on the outcome of human exposure available in the literature, we conclude that while humans face the risk of veno-occlusive disease and childhood cirrhosis PAs are not carcinogenic to humans. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

1.1. Plants containing pyrrolizidine alkaloids

Pyrrolizidine alkaloids (PAs) are found in plants of widespread geographical distribution. Over 200 alkaloids have been identified in 300 plant species of up to 13 families [1,26,75]. It has been estimated that up to 3% of the world flowering plants contain toxic

PAs [1]. The main sources of toxic alkaloids are the families—Boraginaceae (all genera), Compositae (tribes *Senecionae* and *Eupatoriae*) and Leguminosae (genus *Crotalaria*) [2]. Within these families, toxic alkaloids are found mainly in the *Senecio* (Compositae), *Crotalaria* (Leguminosae) and *Heliotropium* (Boraginaceae) species.

PAs are esters of hydroxylated 1-methylpyrrolizidines. Hepatotoxic PAs are esters of unsaturated necines having a 1,2 double bond. The structure of PAs is composed of necine, two fused five-membered rings joined by a single nitrogen atom, and

* Corresponding author. Tel.: +61-7-32749002; Fax: +61-7-32749003; E-mail: a.prakash@mailbox.uq.edu.au

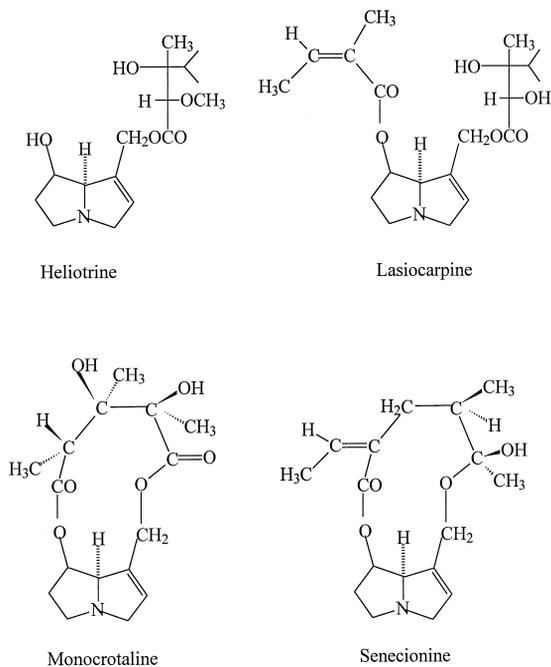


Fig. 1. Representative pyrrolizidine alkaloid structures.

necic acid which is made up of one or two carboxylic ester arms which may form a macrocyclic structure. Fig. 1 shows the structures of some commonly known PAs (Fig. 1).

2. Pathways of human exposure

2.1. Contamination of staple food

Large outbreaks of poisoning have occurred through contamination of wheat crops in Afghanistan, India and the former USSR [3–5]. This is made possible because the PA containing plants, in particular, three species of Boraginaceae, *Heliotropium lasiocarpum*, *H. popovii* and *H. europaeum*, are well adapted to vigorous growth under the climatic conditions in which wheat is usually grown.

2.2. Milk

Laboratory studies have shown PAs are present in milk from cows [6] and goats [7]. Eastman [8] showed

that much of the PAs were excreted in the skim-milk fraction and deduced that these may be water-soluble metabolites. Later, water-soluble PA N-oxides were identified in milk [9]. Milk from lactating rats fed PAs were shown to be mutagenic in the *Salmonella*/mammalian-microsome mutagenicity test [10] and in the *Drosophilla* sex-linked recessive lethal assay [11]. It was also demonstrated that liver lesions (centrilobular megalocytes and biliary ductular hyperplasia) could be elicited in rats consuming milk from lactating goats fed *Senecio jacobaea* (tansy ragwort) [9]. Similar lesions were seen when the rats were directly fed a diet containing 0.001–1% *S. jacobaea*.

2.3. PAs in medicinal plants

PAs have been identified in traditional herbal medicines of South America [12,13], Sri-Lanka [14,15] and China [16,17]. Of the herbal remedies containing pyrrolizidine alkaloids, comfrey has received the most attention. Studies have shown the presence of toxic PAs in fresh leaves [18], commercial comfrey preparations [19] and in comfrey-pepsin capsules [20]. Following an episode of comfrey-pepsin related poisoning [21] the sale of comfrey products for internal use was banned in the US and in Canada [22]. Nevertheless, comfrey leaves and extracts continue to be used in poultices, creams and ointments for topical application. Comfrey leaves are consumed in salads, particularly in Europe, North America, Japan and Australia. Toxic PAs have also been isolated from leaves of *H. digyum* [23], which are consumed in East Africa.

2.4. Honey

The presence of the alkaloids (seneciphyline, senecionine, jacobine, jaconine, jacoline and jacozine) in honey produced by bees foraging in a region of Oregon infested with *S. jacobaea* (tansy ragwort) has been reported [24]. The honey also contained small amounts of ragwort pollen. The PA content was estimated to be at 0.3–3.9 ppm. Similarly, Culvenor et al. [25] found alkaloids (echimidine and smaller amounts of 7-acetyllycopsamine, 7-acetylintermedine, echiumine, uplandicine, lycopsamine, intermedine and acetylechimidine) at 0.54–1.9 ppm in

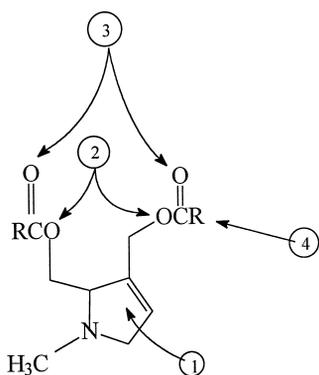


Fig. 2. Structural features essential for PA toxicity. The numbers correspond to the items listed in Section 3.

3. Structural features of PAs

3.1. Features for hepatotoxicity

A comprehensive account of the various naturally occurring PA structures are given by Mattocks [26]. The potential of PA compounds as hepatotoxins is governed by certain minimum structural features: (1) an unsaturated 3-pyrroline ring, (2) one or two hydroxyl groups each attached to the pyrroline ring, (3) one or preferably two esterified groups and (4) the presence of a branched chain on the acid moiety (Fig. 2).

3.2. Steps in the production of toxic metabolite

honey from regions of southeastern Australia where bees forage on *Echium plantagineum* (Patterson's Curse or Salvation Jane).

A schematic representation of the PA metabolic pathway is shown in Fig. 3. This diagram is a

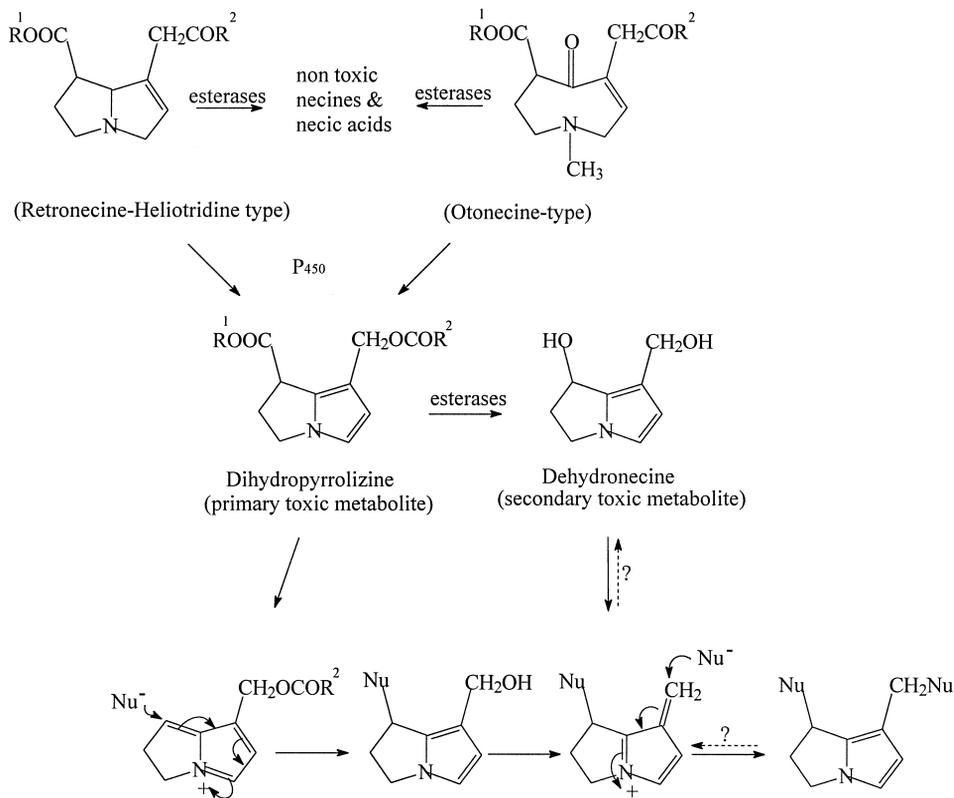


Fig. 3. The metabolic pathway leading to toxicity of pyrrolizidine alkaloids.

modified version of the one published by Roeder [27]. Essentially, the parent PA is either hydrolysed to non-toxic necines and necic acids or to ester pyrroles by esterases or P450 enzymes respectively. The ester pyrroles (EPy) are considered to be hepatotoxic due to their high reactivity while the less reactive longer-lived alcoholic pyrroles (APy), produced by the hydrolysis of the EPy, lead to antimetabolic effects and to mutagenic and carcinogenic lesions.

4. Metabolism of pyrrolizidine alkaloids

There are some hundreds of naturally occurring pyrrolizidine alkaloids but most of what is known about their metabolism and the molecular basis of their toxic effects comes from studies with a limited number of representative compounds with most studies carried out using just a few species of experimental animal. Monocrotaline, senecionine, seniciphylline, jacobine, lasiocarpine, ridelliine and heliotrine appear to have been the most frequently studied alkaloids and rats, pigs, rabbits and guinea pigs have been used most often to study the metabolism and molecular toxicity of these compounds [28,29]. Only a small number of studies have used human organ donor tissue samples or preparations of human tissue enzymes to characterise these biotransformations as they pertain specifically to people.

The parent alkaloid is chemically unreactive. Once ingested, much of it is excreted unchanged but the remainder is metabolised in the liver. Activation requires the dehydrogenation of PAs to pyrroles (Fig. 3) which are electrophilic and will react with nucleophilic tissue components such as nucleic acids and proteins. Since the liver is the site of toxic pyrrole production, it is one of the two main target organs, the lungs being the other.

There are three principal routes by which these compounds are metabolised, with liver being the predominant organ in which most metabolism occurs although small but insignificant contributions from lung and kidney also having been identified. One route of metabolism involves esterase cleavage which releases the necine and necic acid moieties neither of which are toxic or undergo further metabolism. This

appears to be predominantly carried out in the liver but the particular esterase isoforms responsible appear not to have been extensively characterised. This metabolic route is very important after exposure to these compounds because esterase cleavage is a detoxication pathway, promoting the clearance of these xenobiotics as non-toxic products. Esterase activity towards monocrotaline is particularly high in guinea pig liver [30] and this is regarded as one reason for the marked resistance to the toxic effects of this and most other pyrrolizidine alkaloids characteristic of this species. One exception is jacobine which is toxic to guinea pigs, and guinea pig liver microsomes and purified liver carboxylesterases have been shown not to be active in the hydrolysis of jacobine [31]. Rat liver microsomes have zero esterase activity towards monocrotaline and rats are accordingly susceptible to the toxic actions of this compound [30]. Esterase activity of human tissues directed towards pyrrolizidine alkaloids appears not to have been assessed.

A second route of metabolism of these alkaloids is formation of the N-oxide derivative by microsomal monooxygenases. N-oxide formation is another detoxication route whose importance varies widely between species and it appears that differential substrate selectivities of multiple enzymes in different organs are responsible for this variation. The monooxygenases generally found to be most important in this biotransformation are the liver microsomal flavin monooxygenases. In pig liver for example these enzymes are very active in the N-oxidation of senecionine [32] but the corresponding enzymes do not contribute greatly to this biotransformation in rat liver [33]. Purified flavin monooxygenase from rabbit lung was also shown to be inactive in this oxidation reaction [32]. By contrast the flavin monooxygenases of guinea pig lung, liver and kidney microsomes were presumptively identified to be very active in catalysing senecionine N-oxidation [34] and this is recognised as another important factor that explains this species' resistance to the toxic effects of this alkaloid. Although recent studies have contributed greatly to understanding the molecular genetics underlying the diversity of different isoforms of the flavin monooxygenases in various organs and tissues of a number of species there is as yet no clear indication as to the molecular basis of substrate

selectivity of the different forms for any of the pyrrolizidine alkaloids.

A number of reports show that microsomal cytochrome P450 monooxygenases may also be responsible for catalysing the N-oxidation of some pyrrolizidine alkaloids in some species. In rats for example senecionine N-oxidase activity is markedly gender differentiated, this conversion occurring much more rapidly in male than in female animals, and this was attributed to the higher activities of the 'male specific' liver cytochrome P450 isoform UT-A. Another CYP isoform, PCN-E, induced by treatment of the animals with the anti-glucocorticoid pregnenolone 16 α -carbonitrile was also active in this regard [32].

These rat liver enzymes are now identified in the nomenclature system based on primary amino acid sequence homology as CYP2C11 and CYP3A1 (<http://drnelson.utmem.edu/nelsonhomepage.html>). A CYP isoform in the 2C subfamily isolated from guinea pig liver also showed N-oxidase activity towards senecionine [35]. The human liver enzyme CYP3A4 has also been identified as having senecionine N-oxidase activity [36]. The abundance of this enzyme varies widely between individuals which suggests that interindividual variation in clearance of this alkaloid may be very variable but this would also depend on the substrate selectivities of the human flavin monooxygenases which appear to be undetermined.

Conversion of PAs to reactive toxic pyrrolic metabolites is now well established to be due to α -carbon oxidation (dehydrogenation) catalysed by cytochrome P450 monooxygenases [28]. The primary oxidised metabolites are reactive and undergo spontaneous conversion to electrophilic species which can undergo Michael addition reactions with cellular nucleophiles. The sacrificial (protective) nucleophile reduced glutathione (G-SH) traps some of these reactive products as a detoxication route to their clearance but critical protein and nucleic acid nucleophiles also react yielding adducts which have been proposed to cause cell toxicity (see Section 8).

Rat, human, and guinea pig tissues and enzyme preparations have been most commonly used to characterise the enzymes catalysing these bioactivation (toxication) reactions with the most commonly identified isoforms being in the CYP3A subfamily

with 2B and 2D isoforms also having this activity. In human liver microsomes early immunochemical inhibition studies, using antibodies raised in rabbits against purified rat liver CYP isoforms, suggested the debrisoquine 4'-hydroxylase contributes significantly to oxidation of lasiocarpine and monocrotaline [37] and more recently strong evidence has accumulated showing the role of CYP3A4 in toxication of senecionine by the dehydrogenation pathway [36]. Importantly, CYP3A4 was also shown to be able to catalyse senecionine N-oxide formation [36], which strongly implicates this single enzyme as simultaneously catalysing toxication and detoxication of this alkaloid in exposed individuals. Interestingly, the abundance of this enzyme in liver varies over a 30-fold range between individuals which confound attempts to make predictions regarding the rate and extent of metabolism of this alkaloid by either pathway in any individual. A similar dual role (toxication and detoxication) for the mixture of CYP3A subfamily enzymes in male rat liver induced by dexamethasone treatment had been reported previously with senecionine metabolism [33] and equivalent findings with female rats treated with spironolactone (which also induced CYP3A subfamily isoforms) have also been reported more recently, again with senecionine as the alkaloid under study [38]. In a recent paper, rat CYP3A enzymes were also identified as catalysing ^{14}C labeled monocrotaline bioactivation which resulted in covalent binding of ^{14}C to liver microsomes [39].

5. Toxicity in animals

5.1. Livestock poisonings

Although grazing animals do not naturally forage on PA containing plants, they are consumed in drought periods when other food is in short-supply or if the feed-stock is contaminated. Substantial differences in susceptibility occur between animals of different species. Pigs and poultry are most susceptible, while horses and cattle are less so but sheep and goats are relatively resistant to PA toxicity. These differences are believed to be due partly to the variations in the efficiency with which liver enzymes metabolise the parent alkaloid to the toxic pyrrolic

and with respect to sheep, partly by enzymes in the rumen [40].

Several workers have reported PA poisoning of pigs, poultry and ducks in Australia [41–43]. In the most recent case, Gaul et al. [44] recorded a contamination of feed stocks with poisoning in pigs, poultry and calves in southern Australia when higher than average summer rainfall aided the growth of heliotrope weed in wheat fields.

Serious outbreaks of PA poisoning in cattle have occurred throughout the world [45–47]. These epidemics generally occur after a period of high winter rainfall followed by a dry summer, conditions which favour the growth of PA containing weeds in the grazing pasture. There have also been sporadic cases of poisoning due to the contamination of hay with leaves and seeds of toxic plants [48,49]. Calves and young animals show higher susceptibility than older cattle, most of the animals involved in the epidemic in 1994 being less than 3 years old [46]. PA poisoning has been reported in yaks in Bhutan [50]. In addition to the other characteristic features of PA toxicity these animals suffered from skin lesions with hyaline parakeratosis. Sulphur bound pyrrolic adducts were demonstrated in formalin fixed liver [51] and bound to haemoglobin in the circulating blood [52] of affected animals.

5.2. Toxicity in laboratory animals

5.2.1. Lung toxicity

Pulmonary lesions produced by PAs have been extensively investigated, mainly in rats, but also in non-human primates. In one study, dogs dosed with 60 mg/kg of monocrotaline by body weight produced ultrastructural changes in endothelial cells of the alveolar capillaries, prominent accumulation of platelets and the appearance of interstitial oedema [53]. Similar lesions were observed in Sprague–Dawley rat lungs using a single subcutaneous injection of monocrotaline at 60 mg/kg by body weight [54].

5.2.2. Carcinogenicity

While there is no evidence of cancer in the literature concerning domestic animals exposed to PAs, studies carried out under laboratory conditions have been able to produce PA-induced cancer in rodents.

Some of the plant species known to cause cancer in rodents are *S. longilobus*, [55] *Petasites japonicus* Maxim [56], *Tussilago farfara* L. [57], *Symphytum officinale* [58], *Farfugium japonicum* [59], *Ligularia dentata* [60] and *S. cannabifolis* [61]. Further, individual PA compounds such as monocrotaline [62,63], heliotrine [64], lasiocarpine [65,66], clivorine [60], petasitenine [67] and riddelliine [68] have also been shown to be carcinogenic in experimental animals.

6. Human poisonings

6.1. Hepatic veno-occlusive disease (VOD)

VOD characterised by epigastric pain with abdominal distension due to ascites has been associated with human consumption of PAs often by the accidental contamination of grain with seeds containing PA or through herbal remedies. Some of the most serious outbreaks of PA poisoning were reported in NW Afghanistan [69] and central India [4] following a severe period of drought, during which heliotropium plants were seen to thrive in the region. The staple food was contaminated with seeds of the *H. popovii* plant. The most recent outbreak occurred in 1992 in Tadjikistan [5]. These seeds contained the PAs, heliotrine and lasiocarpine. VOD was endemic in regions of South America during the latter part of the century [70] but with better education on the proper identification of plants this is no longer the case. However, sporadic cases are still being reported from around the world [71–73].

6.2. Teratogenicity

VOD has been reported in an infant born to a woman who had consumed herbal tea brewed from the leaves of *T. farfara* (coltsfoot) which contained 0.6 mg/kg senecionine by dry weight [74,75].

At least one of the components in *S. madagascariensis*, an introduced species, which has spread over vast regions of coastal South East Queensland in Australia seems to have high lipophilicity and it is suggested that this may enable it to cross the placenta and cause hepatic failure in the foetus. This view is supported by the recent observation of pyrrolizidine alkaloidosis in a two-month-old foal

caused by consumption of *S. madagascariensis* by the mother [76].

6.3. Carcinogenicity

Schoental's group which showed the formation of primary liver tumours in rats following feeding of alkaloids [77] first raised the possibility that PAs may also play a role in human carcinogenesis [78]. Since then several PAs and their metabolites have been shown to be carcinogenic in rodents. However, though there are several recorded cases of human exposure to PAs, with exposure levels ranging from acute to chronic levels, there exist no reports to date of cancer associated with such exposures.

7. Toxicology of pyrrolizidine alkaloids

The classic feature of chronic PA poisoning is VOD, hepato-splenomegaly and emaciation. After the liver, the lungs are the next most common sites of PA toxicity. Pyrroles formed by the metabolism in the liver can travel to the lungs. Initial changes seen in the pulmonary vasculature included thrombi in vessels, acute inflammation and thickening of vessel walls leading to occlusion. These effects along with the interalveolar septal fibrosis lead to pulmonary hypertension. The result of the impaired pulmonary blood flow is increased work for the right ventricle causing it to hypertrophy and eventually leading to congestive heart failure [79].

Other important chronic toxicities of PAs are antimetabolic effects observed in rodents and domestic animals and cancer reported only in rodents.

7.1. Antimetabolic activity

One of the characteristic features of chronic pyrrolizidine alkaloid poisoning in animals is the development of enlarged cells, or megalocytosis [80]. Megalocytes appear to be the result of a combined action of PAs on the hepatocytes, a stimulus to regenerate following parenchymal cell injury, and the antimetabolic action of the pyrrole metabolites of PAs [26]. Post-mortem examinations of cattle [81] horses [81,82] and yaks [51] which have died after consuming PA containing plants have revealed the

presence of hepatic megalocytes. Field experiments have demonstrated megalocytosis in the livers of livestock fed PA containing plant material [10,83,84]. In addition megalocytes have been observed in experimental animals, rats [7,85,86], mouse, sheep, horse, pig and most recently in the chick embryo [87]. Mattocks [26] suggested that these cells appear within a few weeks and this lesion may persist for the lifetime of the animal. However, recently it was shown that transplantation of normal hepatocytes into rats treated with lasiocarpine significantly reduced the number of existing megalocytes [86].

Megalocytosis has been demonstrated in mammalian cell culture also. It was seen in cultured bovine pulmonary artery endothelial cells (BEC) exposed to a monocrotaline pyrrole (MCTP) [88]. Kim et al. [89] were also able to demonstrate megalocytosis in a bovine kidney epithelial cell line exposed to a range of alkaloids. Megalocytosis can be produced by a single sublethal dose [90] or by a cumulative effect of small doses [91].

Megalocytosis has been found in other organs such as kidney and lungs as well [92]. It has been shown to occur in cultured human fetal liver cells [93] but has never been observed in the affected human livers [3].

It is believed that the formation of megalocytosis is a result of the action of the metabolite pyrrole ester [26]. It is thought that the pyrrole disrupts the cell cycle by damaging key genes which control cell division leading to mitotic bypass [94]. Section 9 addresses the question of PA effects on the cell cycle in greater detail.

7.2. Genetic toxicology of pyrrolizidine alkaloids

Clark was able to classify several PAs according to their mutagenicity in *Drosophila melanogaster* [95]. Monocrotaline, lasiocarpine and heliotrine (see Fig. 1) showed strong mutagenic property in this assay. Numerous other studies have demonstrated the mutagenicity of PAs [96–99]. Milk from lactating rats fed PAs was shown to be mutagenic in the *Salmonella*/mammalian-microsome mutagenicity test [10]. Recently, Berry et al. [100] used a primary hepatocyte-mediated V79 cell mutagenesis and DNA-repair assay system to study the genotoxic effects of PAs. Based on their results they concluded

that riddelliine and structurally related PAs are likely to be hepatocellular carcinogens as well as cytotoxic agents.

8. Interactions of pyrrolizidine alkaloids with cellular components

The highly electrophilic nature of PA metabolites suggests that they would react readily with nucleophilic tissue constituents such as DNA and proteins. The earliest observation of PA interacting with DNA was reported by White et al. [101]. Pyrroles alkylate proteins as well [102]. Chromatography, NMR and spectral analysis were used to show interaction between the C-7 position of dehydroretronecine (DRN), a metabolite of monocrotaline, and the sulfhydryl groups of cysteine and glutathione. Alkylation between the C-7 of DRN and the exocyclic amino group (NH₂) of deoxyguanosine (dG) has also been demonstrated [103]. Even though DRN is a bifunctional alkylating agent only monoadducts with equimolar quantities of DRN and dG were detected and the C9 was less reactive than the C7 position in this study. In vitro studies carried out in our lab showed that dehydromonocrotaline (DHM) alkylated N7 guanine in a sequence selective fashion [104]. Further, we also found evidence that DHM formed polymers at sub millimolar concentrations which induced multiple DNA fragment crosslinks, a phenomenon never observed before with any other class of DNA crosslinking agents. Polymer formation by bifunctional PA metabolites in cells has not yet been reported, but Mattocks [26] predicted its formation and discussed the relevance of these structures under biological conditions.

Petry et al. [105] used the alkaline elution technique to show dose dependent DNA–DNA interstrand and DNA–protein crosslinks in hepatic nuclei of Sprague–Dawley rats treated intraperitoneally with 5–30 mg monocrotaline/kg body weight. The alkaline elution was also used to determine crosslinking in cultured porcine pulmonary artery endothelial cells (PEC) exposed to an unspecified monocrotaline pyrrole (MCTP) [106]. DNA–DNA and DNA–protein crosslinking were seen 4 h after exposure to MCTP and the degree of crosslinking increased till the medium was changed at 48 h. By this time the

DNA–protein crosslinking was significantly greater than DNA–DNA crosslinking. Despite replacement with fresh medium lacking MCTP every two days, the crosslinking factor remained elevated till day 10. Hinks et al. [107] also showed DNA–protein interactions predominate over DNA–DNA ones in cultured Madin Darby bovine kidney (MDBK) epithelial cells exposed to a range of eight bifunctional alkaloids in the presence of an external metabolising system. This study also showed a correlation between crosslinking ability and suppression of colony formation that strengthens the hypothesis that crosslinking is involved in the biological activity of PAs. Some of the most potent crosslinkers were able to inhibit colony formation altogether. Later this group demonstrated megalocytosis in cultured cells exposed to these alkaloids [89] (see below). The most recent work in MDBK cells [108] suggests a DNA–PA–protein structure. The protein isolated had a molecular weight of 40–60 kDa and a net acidic charge (isoelectric point 4.2–5.0). The authors suggest it may be actin (pI 5.4, molecular weight 45 kDa).

9. Effect of pyrrolizidine alkaloids on the cell cycle

In yeast, damage to DNA results in cell cycle arrest at one of the checkpoints, enabling DNA repair. This happens in mammals too, however, an additional cell death (apoptosis) pathway may be activated, since the primary aim of multicellular organisms is the survival of the whole, rather than the preservation of individual cells which may foster mutations.

The role of the p53 tumour suppressor gene in the control of the cell cycle at the G1/S stage is well established. It has been suggested that p53 may be involved in controlling the cycle at G2/M phase as well [109]. Other important cell cycle regulatory elements involved in G2/M and M/G1 checkpoints are *rum + 1*, *cdc2*, *cdc25*, cyclin B, and *RMSA-1* (regulator of mitotic spindle assembly, [110]. Recently, Couet et al. [111] observed a specific mutation in codon 249, exon 7 of the p53 gene in a human Chang liver cell line treated with PAs and an external metabolising system. Recent work in our lab [112] using SD rats treated intraperitoneally with

oxygenases to either N-oxides or dehydro PAs or pyrroles [26]. Hydrolysis and N-oxide formation are detoxication reactions and as such are generally without harm to the cell. Dehydro PAs are considered to be the primary toxic metabolites [26] and may react with available nucleophiles within the cell. These ester pyrroles (EPy) may also undergo hydrolysis with the formation of pyrrolic alcohols (APy). These are the secondary toxic metabolites and while they are far less reactive than the ester pyrroles, they are far more persistent.

The alkylating species is thought to be a carbonium ion with its reactive centre at C9. If the C7 position also has an oxygen function then active centres exist at both C7 and C9 with the C7 position the more active. This enables this type of toxic metabolite to act as a bifunctional alkylating agent [26].

The pyrrole once formed within the cell may bind covalently to sulphur, nitrogen and oxygen containing groups on various macromolecules. While the S-bound pyrroles are the most stable and may persist in the cell for a considerable time as protein-bound complexes (Py-SPr), these reactions are by and large reversible. This means that the pyrrole moiety may be released from its bond to protein into the cell as APy with secondary toxicity.

The production of the primary toxic metabolite is followed by reaction mainly with proteins at the site of formation. These pyrroles to varying degrees have persistence in aqueous media which enables them to penetrate into the nucleus and react with DNA causing crosslinking within DNA and between DNA and nucleo-proteins. These reactions lead to immediate damage to the hepatocyte. Reactions with soluble molecules in the cytoplasm such as GSH are, however, protective for the cell. GSH-pyrrole adducts (Py-SG) and other soluble reaction products are eliminated from the cell into the bile and/or sinusoidal blood for excretion into the urine.

Primary toxic metabolites of many PAs are sufficiently persistent as to be able to pass from the hepatocyte into the adjacent Dissé space and into the sinusoidal lumen [118]. Here the pyrroles may attack the associated sinusoidal lining cells such as endothelial cells and also become bound on passing red blood cells [118,119]. Specially long lived primary pyrrolic metabolites may even reach the lungs and

heart where they may cause damage to the macromolecules of these organs [120]. The immediate reactive effects of the primary toxic metabolites are considered to be responsible for the damage to the peri-acinar hepatocytes (because the activating P450 enzymes are concentrated in the cells of this parenchymal zone) and to the associated sinusoidal lining and walls of the small hepatic veins leading to VOD.

With outbreaks of acute PA toxicities in humans it is estimated that about 20% die and some 50% recover completely within a few weeks. Of the remainder some 20% appear to recover clinically but may develop chronic VOD and cirrhosis after several years. Others develop a sub-acute VOD and this may eventually resolve or else progress to chronic VOD and cirrhosis [121]. There are also reports of PA toxicities in domestic animals in which following relatively low doses of the alkaloids, often insufficient to cause acute toxicity, death due to chronic hepatic failure occurs several months or years later [122].

It has been established that following a single dose of a PA, almost all of the compound including its soluble metabolites is eliminated from the body within 24 h [123]. Yet in some individuals such an exposure can lead to a progressive and eventually total hepatopathy [90]. It has been suggested that the presence of reversibly bound pyrrolic metabolites in the hepatocytes and endothelial cells of the liver are responsible for this effect [124]. If re-released pyrroles react with GSH they will be safely removed from the liver over time. If re-released pyrroles go on to bind to vital macromolecules then hepatocellular necrosis and VOD may be sustained. If the pyrroles bind to DNA then mutations either leading to an antimetabolic action and/or cancer may occur. The agents re-released from transiently ineffective macromolecular binding would be the persistent secondary toxic metabolite pyrrolic alcohols. These have the persistence in the body and the potential to reach all parts of the liver cell and its surrounding structures and are considered to be mainly responsible for targeting specific DNA binding sites, leading to the promotion of the megalocytosis in the liver and other tissues, a radiomimetic-type effect [26].

Chronic hepatotoxicity caused by PA exposure will be associated with hepatocellular injury leading

to cirrhosis. Because of the production of persistent electrophilic metabolites that can pass out of the hepatocytes in which they are formed the chronic hepatopathy may be characterised in addition by VOD. This is the pathological picture for chronic liver disease caused by PA in man.

In both laboratory and domestic animals the hepatopathy is also characterised by an antimetabolic effect in that megalocytosis and hepatic atrophy are also present and this in fact may be the dominant pathological feature of the disease in these species [125]. Many PA-containing plants and PAs in addition have been shown to be carcinogenic in rodents, mainly in the liver (adenomas and haemangiomas) [2] and the secondary toxic metabolite dehydroretroecine has been shown to be a directly acting carcinogen in the skin [62]. Notwithstanding the ubiquitous presence of PAs as environmental toxicants and contaminants, on a global basis, cancer has not been recorded in domestic animals or humans as a result of such exposures. Even the antimetabolic effect so characteristic of affected livers in animals is not a feature of the hepatopathy in man [126]. It seems that human hepatocytes are resistant to the potentially genotoxic action of these compounds at least at the dose level to which humans are exposed and which can cause these lesions in animals [126].

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