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Pyrrolizidine Alkaloids - Tumorigenic Components in Chinese Herbal Medicines and Dietary Supplements

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ABSTRACT

Traditional Chinese medicine (TCM) has long been used for treating illness in China and other Asian countries, and recently used by the Western countries in several different ways, either for new drug development, or as functional foods and dietary supplements. However, quality assurance and health adverse effects of the herbal plants have not been well studied. Pyrrolizidine alkaloids, a class of hepatotoxic and tumorigenic compounds, have been detected in herbal plants and dietary supplements. In this review, the sources of the pyrrolizidine alkaloid-containing Chinese herbal plants in China and the toxicity, genotoxicity, and tumorigenicity of these compounds are discussed. The metabolic pathways, particularly the activation pathways leading to genotoxicity, are discussed. Recent mechanistic studies indicate that pyrrolizidine alkaloids induce tumors via a genotoxic mechanism mediated by 6,7-dihydro-7-hydroxy-1-hydroxymethyl-5*H*-pyrrolizine (DHP)-derived DNA adduct formation. This mechanism may be general to most carcinogenic pyrrolizidine alkaloids. Perspectives are included for suggestion of directions of future research.

Key words: Pyrrolizidine alkaloids; Chinese herbal medicines; tumorigenecity

INTRODUCTION

Traditional Chinese medicine (TCM) has been used in China for treating illness for more than 2000 years[(1)]. Many people in other Asian countries have also taken TCM for clinical practice or improving health for a long time. During the last two decades, the use of Chinese herbal medicines in Europe, Australia, and North America for health care has been increasing[(2)]. There are several different usage of the Chinese herbal plants; for new drug development, as natural remedies, functional foods (mixing functional herbs in conventional food), and dietary supplements.

Although there are merits in using TCM, several problems exist. These include poor quality control and assurance of the herbal plants, the lack of scientific evidence for the pharmacological effects, and the possible health adverse effects, such as the induction of herb-drug interaction[(3,4)]. There is an extremely large number of herbal plants used for TCM and many components are present in each of the herbal plants. However, information is limited on the genotoxicity and tumorigenicity of the herbal plants commonly used in TCM as well as natural remedies, functional foods, and dietary supplements. Of particular concern is that the active therapeutic components and/or the other chemicals in an herbal plant may be tumorigenic. In this review, we report that pyrrolizidine alkaloids are a class of hepatotoxic and tumorigenic compounds that have been detected in herbal plants and in several dietary supplements. The sources of pyrrolizidine alkaloid-containing Chinese herbal plants in China, their toxicity, mechanisms of genotoxicity and tumorigenicity and perspectives are presented.

SOURCES OF PYRROLIZIDINE ALKALOID-CONTAINING PLANTS

Pyrrolizidine alkaloids are common constituents of hundreds of plant species of different unrelated botanical families distributed in many geographical regions in the world[(2,5-12)]. It has been reported that about 3% of the world flowering plants contain toxic pyrrolizidine alkaloids[(10)]. Toxic pyrrolizidine alkaloid-containing plants grow in South Africa, Central Africa, West Indies, China, Jamaica, Canada, Europe, New Zealand, Australia, and the United States[(13,14)]. Pyrrolizidine alkaloids are found in more than twelve higher plant families, among which three families, *Boraginaceae, Compositae* (*Asteraceae*), and *Legumionsae* (*Fabaceae*), contain most toxic pyrrolizidine alkaloids. More than 660 pyrrolizidine alkaloids and pyrrolizidine alkaloid N-oxides have been identified in over 6,000 plants of these three families, and about half of them exhibit toxic activities[(2,13)]. Because toxic pyrrolizidine alkaloid-containing plants are widely distributed in the world, the risk to human health posed by exposure to these compounds has been a concern.

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Journal of Food and Drug Analysis, Vol. 10, No. 4, 2002

ROUTES OF HUMAN EXPOSURE TO PYRROLIZIDINE ALKALOIDS

I. *As food and food contaminants*

Pyrrolizidine alkaloids have been found to be contaminants of human food sources, such as wheat, milk, honey, herbal medicines, and herbal teas, and this may potentially cause worldwide human health problems[5,8,15-22]. Large scale food poisonings by contamination with toxic pyrrolizidine alkaloids have been reported in several countries[9,13,22,23]. It occurred as early as 1920 when an incidence of large scale food poisoning in South Africa was associated with consumption of bread made from wheat flour contaminated with toxic pyrrolizidine alkaloids[24]. Serious food poisoning occurred again in South Africa in 1950[24,25], in Afghanistan and India in 1970s[7,26,27], and in Tadjikistan in 1992[23].

The intake of products derived from animals grazing on plants containing toxic pyrrolizidine alkaloids is another route of food contamination. People in Europe, North America, Japan, and Australia frequently consumed the leaves of comfrey in salads in the past[22]. A number of pyrrolizidine alkaloid-containing plants have been used as vegetables in Japan, including Senecio cannabifolius, *Petasites japonicus, Tussilago farfara, Farfugium japonicum, and Symphytum officinale*[28]. *Senecio jacobaea*[29] and *Echium plantagineum*[30] were consumed as food in Oregon and Southeastern Australia, respectively.

II. *As herbal teas and herbal medicines*

The intake of herbal medicines and herbal teas has been the most worrisome route of human exposure to pyrrolizidine alkaloid-containing plants[8,9,31-37]. Folk teas used for medicinal purposes have been quite popular in many underdeveloped and developed countries including South Africa, India, Japan, China, Jamaica, Mexico, Europe, South America, Sri-Lanka, and the United States. However, many of these folk teas contain toxic and tumorigenic pyrrolizidine alkaloids[2,8,13,22].

PYRROLIZIDINE ALKALOIDS IN CHINESE HERBAL PLANTS

Since the early 1960s, there are some papers published in the Chinese literature reporting the finding of pyrrolizidine alkaloids in Chinese herbal plants[38-40]. However, more papers were published elsewhere, mainly by scientists from the other countries, particularly from the laboratories of Roeder and Edgar (reference 2 and references cited in). In 1992 Edgar *et al.*[41] estimated that possibly over 50 Chinese herbal plants contain pyrrolizidine alkaloids. Roeder and co-workers have extensively studied plants containing pyrrolizidine alkaloids produced in many regions of the world, particularly in Europe[8] and China[2], and contributed significantly in this field. In his recent review entitled "Medicinal plants in China containing pyrrolizidine alkaloids" Roeder described in detail the findings on this subject and indicated that there are about 90 pyrrolizidine alkaloids found in 38 TCM herbs[2]. The finding is in agreement with that reported in a Chinese article in 1998 by Zhao *et al.*[39].

Table 1. List of the family, genus and species of the pyrrolizidine alkaloid-containing Chinese herbal plants so far identified in China.

Family	Genus	Species
1. Orchidaceae	*Liparis*	1. *Liparis nervosa*
2. Fabaceae	*Crotalaria*	2. *Crotalaria albida*
		3. *C. assamica*
		4. *C. mucronata*
		5. *C. sesseliflora*
		6. *C. tetragona*
3. Boraginaceae	*Arnebia*	7. *Arnebia euchroma*
	Cordia	8. *Cordia myxa*
	Cynoglossum	9. *Cynoglossum amabile*
		10. *C. lanceolatum*
		11. *C. officinale*
		12. *C. zeylanicum*
	Heliotropium	13. *Heliotropium indicum*
	Lappula	14. *Lappula intermedia*
	Lithospermum	15. *Lithospermum erythrorizon*
4. Asteraceae	*Ageratum*	16. *Ageratum conyzoides*
(Compositae)	*Chromolaena*	17. *Chromolaena odorata*
	Eupatorium	18. *Eupatorium cannabinum*
		19. *E. chinense*
		20. *E. fortunei*
		21. *E. japonicum*
	Cacalia	22. *Cacalia hastata*
		23. *C. hupehensis*
	Crassocephalum	24. *Crassocephalum crepidioides*
	Emilia	25. *Emilia sonchifolia*
	Farfugium	26. *Farfugium japonicum*
	Gynura	27. *Gynura bicolor*
		28. *G. divaricata*
		29. *G. segetum*
	Ligularia	30. *Ligularia dentata*
		31. *L. hodgsonii*
		32. *L. intermedia*
		33. *L. lapathifolia*
		34. *L. lidjiangensis*
		35. *L. cymbulifera*
		36. *L. duiformis*
		37. *L. vellerea*
		38. *L. tongolensis*
		39. *L. tsanchanensis*
		40. *L. heterophylla*
		41. *L. platyglossa*
	Petasites	42. *Petasites japonicus*
	Senecio	43. *Senecio argunensis*
		44. *S. chrysanthemoides*
		45. *S. integrifolius var. fauriri*
		46. *S. nemorensis*
		47. *S. scandens*
	Syneilesis	48. *Syneilesis aconitifolia*
	Tussilago	49. *Tussilago farfara*

Table 2. List of the tumorigenic pyrrolizidine alkaloid-containing Chinese herbal plants grown in China[1].

Plant	Herb name in Chinese	Medicinal purpose	Tumorigenic pyrrolizidine alkaloids	References
1. Family Fabaceae				
Crotalaria assamica Benth	Zi xiao rong (Nung gi li)	Folk remedy	Monocrotaline	43
Crotalaria mucronata	Zhu zi tou	Folk medicine	Monocrotaline, retrorsine	1
Crotalaria sessiliflora L . or *Crotalaria assamica* Benth	Ye bai he	Folk medicine	Monocrotaline	43
2. Family Boraginaceae				
Heliotropium indicum L .	Da wei yao	Ulcer, wounds and local inflammations	Heliotrine, lasiocarpine	2
Lappula intermedia M. Popov.	He shi	Ascariasis, oxyuriasis, infantile malnutition	Lasiocarpine	2
Lithospermum erythrorhizon Sieb. et Zucc.	Zi cao	Antipyretic and antiphlogistic	Intermidine	7
3. Family Asteraceae (Compositae)				
Ageratum *conyzoides L.*	Sheng hong ji	Common colds, fever, malaria	Lycopsamine	44
Chromolaena odorata R. M. King & H. Rob.	Fei ji cao	Hemostatic	Intermidine	7
Eupatorium cannabinum L .	Pei lan	Influenza, cerebral stroke	Lycopsamine, intermidine	7,44
Eupatorium japonicum Thunb.	Hua zhe lan and o Cheng gan ca	Measles, rheumatic bone pains and colds	Lycopsamine, intermidine	7,44
Crassocephalum crepidioides *S. Moore*	Jia tong hao	Cold, dysentery, gastroenteritis,	urinary infection Jacobine	2
Emilia sonchifolia DC	Yang ti cao, Yi dian hong	Antipyretic, diarrhea, hemoptysis	Senkirkine	2
Farfugium japonicum Kitam	Lian peng cao	Colds and flu	Petasitenine, senkirkine	2
Gynura bicolor DC	Guan yin xian	Dysmenorrhea, tuberculous hymoptysis	Retrorsine	47
Gynura segetum Merr.	Ju shan qi, Tu san chii	Hemoptysis, peripheral blood circulation disorder	Senecionine,seneciphylline	2,46
Ligularia hodgsonnii Hook		Antitussive	Clivorine	42
Senecio argunensis Turcz.	Yu yie qian li guang, Zhan long cao	Folk medicine, dysentery	Senecionine, seneciphylline	2
Senecio chrysanthemoides DC	Chien li kuang, Tsang tu san chi	Traumatic injury, breast abscesses	Seneciphylline	2
Senecio nemorensis	Huana wan	Enteritis, hepatitis, boils	Senecionine	2
Senecio scandens	Quian li guang, Chiu li ming	Oral and pharyngeal infection	Senecionine, seneciphylline	2
Tussilago farfara L .	Kuan dong hua, Chien hua	Chronic bronchitis, asthma, influenza	Senecionine, senkirkine	2

1: Most of the data obtained from[2]. In most cases, the major tumor type is hepatocarcinoma.

The most recent study[40] by Lin *et al.* in Hong Kong and Wang *et al.* from Nanjing, China has identified additional 11 Chinese herbal plants containing pyrrolizidine alkaloids that were not indicated in Roeder's review[2]. These plants all belong to *Ligularia* genus of the *Asteraceae* (*Compositae*) family. Thus, a total of 49 species of the Chinese herbal plants containing pyrrolizidine alkaloids have been identified. All these plants are listed in Traditional Chinese Dictionary published in 1979[38] and used as TCM herbs in China. The families, genera, and species of the pyrrolizidine alkaloid-containing Chinese herbal plants so far identified in China are summarized in Table 1. Among these plants, one plant belongs to the *Orchidaceae* family, five to the *Fabaceae* (*Leguminosae*) family, nine to the *Boraginaceae* family, and 37 to the *Asteraceae* (*Compositae*) family. The geographic distribution of these plants grown in China is shown in Figure 1.

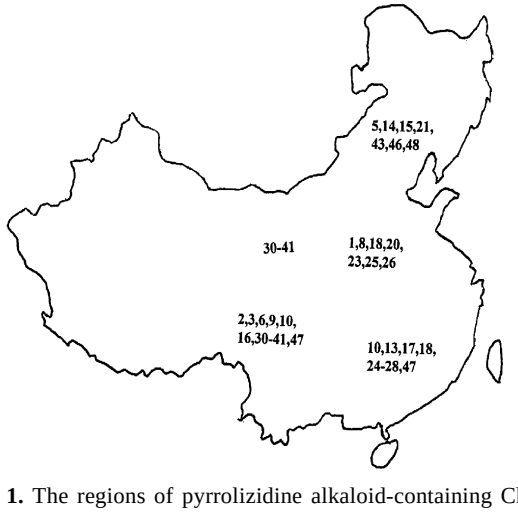

Figure 1. The regions of pyrrolizidine alkaloid-containing Chinese herbal plants in China. See Table 1 for legend to specific plants.

Journal of Food and Drug Analysis, Vol. 10, No. 4, 2002

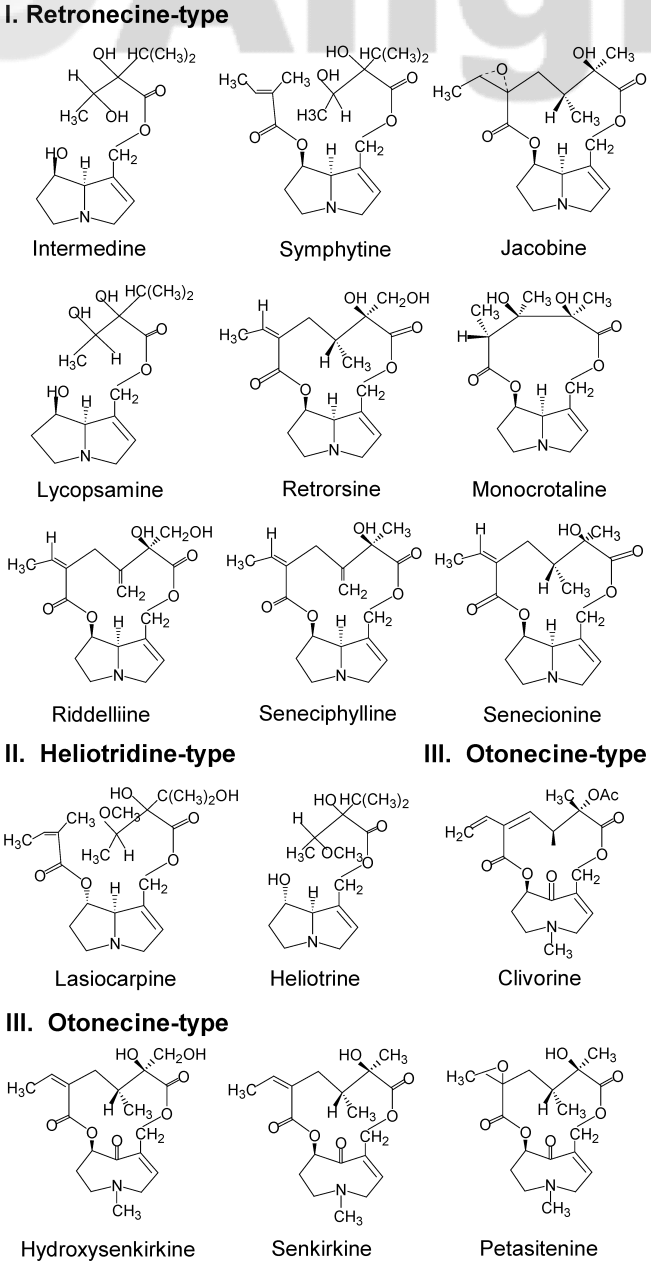

Figure 2. The names and structures of tumorigenic pyrrolizidine alkaloids identified in Chinese herbal plants in China.

The herbal plants listed as *11*, *12*, *19*, *42*, and *49* are grown widely spread in many regions in China and therefore their locations of production are not shown in Figure 1. It is quite common that various plant sources for the same herbs are found in different locations. For example, the plant sources for a TCM herb Bei Mu (Bulbs Fritillaria) can be found in more than 25 provinces in China.

Among 49 pyrrolizidine alkaloid-containing Chinese herbal plants produced in China, there are only 5 plant species that are documented to be officially used as the plant sources for TCM herbs in the latest edition of China Pharmacopoeia (2000 Edition). They are *Lithospermum erythrorhizon* (Zi Cao), *Arnebia euchroma* (Ruan Zi Cao), *Eupatorium fortunei* (Pai Lan), *Senecio scandens* (Qian Li Guang), and *Tussilago farfara* (Kuan Dong Hua). The remaining 44 pyrrolizidine alkaloid-containing Chinese herbal plants are used as the plant sources for the locally used TCM herbs in different areas in China as documented in Traditional Chinese Dictionary[38].

Among the more than 90 pyrrolizidine alkaloids identified from these herbal plants in China, 19 herbal plants have been found to induce tumors in experimental animals. There are 15 tumorigenic PAs identified in these 19 herbal plants. The names and structures of these 15 compounds are shown in Figure 2. The names of the herbal plants that contain the tumorigenic pyrrolizidine alkaloids and their medicinal purposes are listed in Table 2. However, it is highly possible that the genotoxicity, particularly tumorigenicity, of the other pyrrolizidine alkaloids in these Chinese herbal plants has not been systematically studied. Base on the structure-activity consideration, we estimate that at least another 40 pyrrolizidine alkaloids from these Chinese herbal plants are genotoxic and possibly tumorigenic. The names and structures of some of these pyrrolizidine alkaloids are shown in Figure 3.

It is highly possible that many more Chinese herbal plants may contain pyrrolizidine alkaloids but have not yet been well characterized. As an example, Lin *et al.*[42] recently reported that two otonecine-type pyrrolizidine alkaloids, clivorine and ligularine, are present in *Ligularia hodgsonnii* Hook (Compositae family), an antitussive traditional Chinese medicine produced in rural areas of Sichuan providence, China. Clivorine has been shown to induce tumors in

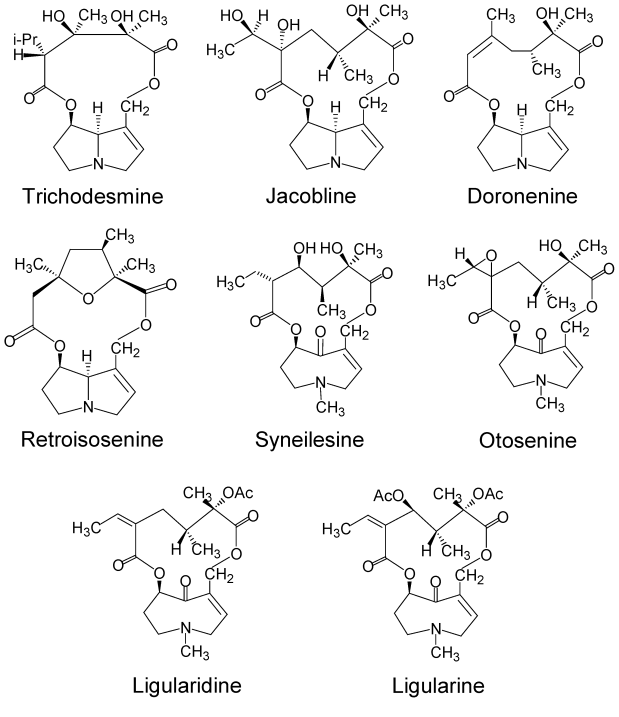

Figure 3. The names and structures of suspected genotoxic or tumorigenic pyrrolizidine alkaloids identified in Chinese herbal plants in China.

rats[42]. Compositae (Asteraceae) is the largest family of plants in the world as well as in China. The Compositae family consists of more than 900 genera and 20,000 species. *Ligularia* is one genus but, as described above, the recent study by Zhao *et al.*[40] identified 11 species from *Ligularia* that contain pyrrolizidine alkaloids. This finding well implicates that there should be many more pyrrolizidine alkaloid-containing Chinese herbal plants grown in China. Thus, it is highly likely the identification of pyrrolizidine alkaloids in Chinese herbal plants in China has never been intensively pursued. As such, human health risk posed by consumption of pyrrolizidine alkaloid containing Chinese herbal plants and the products (dietary supplements and functional foods) made from these Chinese herbal plants is a serious concern.

PYRROLIZIDINE ALKALOIDS CONTAINED IN FUNCTIONAL FOODS AND DIETARY SUPPLEMENTS

During the last two decades, the use of medicinal plants has rapidly increased in Europe, North America, and Australia[2,8]. Unfortunately, as Roeder[7] reported in 1995, many pyrrolizidine alkaloid-containing medicinal plants were consumed in Europe. Some of these herbal plants might have been sold as dietary supplements. Among pyrrolizidine alkaloid-containing herbal plants, 18 plants have been shown to induce tumors in experimental animals, including: (i) in Family *Boraginaceae: Anchusa officinalis* L., *Borago officinalis* L., *Heliotropium arborescens* L., *Myosotis scorpioides* L., *Symphytum asperum* Lepech, *Symphytum caucasicum* Bieb., *Symphytum officinale* L. (Comfrey), and *Symphytum uplandicum* Nyman (Russian comfrey); and (ii) in Family *Asteraceae* (*Compositae*): *Eupatorium cannabinum* L., *Adenostyles alliariae* Kern, *Emilia sonchifolia* DC., *Petasites hybridus* PH Gaertn., B., Mey & Scherb. (Colts food), *Petasites spurius* RCHB, *Senecio bicolor* Tod. ssp. *cineraria*, *Senecio jacobaea* L. (Tansy ragwort, European ragwort), *Senecio nemorensis* L. ssp., *Senecio vulgaris* L., and *Tussilago farfara* L. (Coltsfoot)[8]. Consequently, these toxic medicinal plants have been either banned or limited in use.

Pyrrolizidine alkaloid-containing herbal plants, including comfrey, coltsfoot, and borage, have been sold as dietary supplements[2,8,12,15,17,18]. Comfrey and coltsfoot are Chinese herbal medicine and produced in many countries including China, and borage is produced in Chile, Mexico, France, Spain, Turkey, and USA. It is not known whether pyrrolizidine alkaloids are present in any of the other commercial dietary supplements. Since the use of dietary supplements and functional foods has increased rapidly in the world, the risk of human exposure to toxic pyrrolizidine alkaloids by taking dietary supplements requires to be assessed.

TOXICITY OF PYRROLIZIDINE ALKALOIDS

Pyrrolizidine alkaloids require metabolic activation to exert their acute and chronic toxicity. Livestock and human poisoning upon consuming pyrrolizidine alkaloid-containing plants have been reported in many areas of the world. The poisoned animal species include horses, cattle, sheep, goats, swine, chickens, quails, and doves[47,48]. Acute poisoning causes massive hepatotoxicity with hemorrhagic necrosis. Chronic poisoning takes place mainly in liver, lungs, and blood vessels, and in some instances kidneys, pancreas, gastrointestinal tract, bone marrow, and brain. Exposure over a longer period of time causes cell enlargement (megalocytosis), veno-occlusion in liver and lungs, fatty degeneration, nuclei enlargement with increasing nuclear chromatin, loss of metabolic function, inhibition of mitosis, fatty degeneration, proliferation of biliary tract epithelium, liver cirrhosis, nodular hyperplasia, and adenomas or carcinomas[8].

Pyrrolizidine alkaloids have been determined to exhibit a large variety of genotoxicity, including DNA binding, DNA cross-linking, DNA-protein cross-linking, sister chromatid exchange, chromosomal aberrations, mutagenicity, and carcinogenicity[5-7,16,28,49-68]. A number of pyrrolizidine alkaloids, including clivorine, heliotrine, lasiocarpine, retrorsine, senkirkine, seneciphylline, and riddelliine, were found to be mutagenic in *Salmonella typhimurium* TA100 in the presence of the S9 activation enzyme system[69-72]. Pyrrolizidine alkaloids are among the first naturally occurring carcinogens identified in plants[7]. The 19 tumorigenic pyrrolizidine alkaloids detected in Chinese herbal plants in China are shown in Figure 2. The experimental procedures for the *in vivo* animal studies, including the section of animal species and strain, route of chemical administration, and time duration, have been well described in the literatures[6,,28,49-57].

Pyrrolizidine alkaloids consist of a necine base and a necic acid. The necic acids are four to six carbon-containing mono- or di-carboxylic acids. It has been found that most of the pyrrolizidine alkaloids that exhibit toxic effects are derived from esters of basic alcohols, the necine bases, and with a double bond between the C1 and C2. The structures and numbering system of the four types of representative

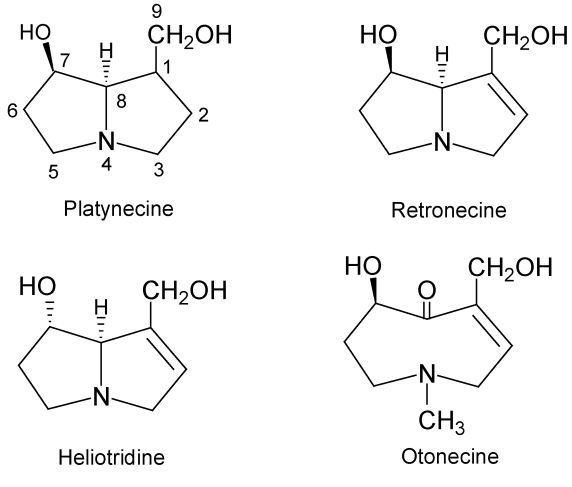

Figure 4. The common necine bases of pyrrolizidine alkaloids.

Journal of Food and Drug Analysis, Vol. 10, No. 4, 2002

necine bases, platynecine, retronecine, heliotridine, and otonecine are shown in Figure 4. Thus, the platynecine type pyrrolizidine alkaloids, which do not contain a double bond in the necine base, are considered either weakly toxic or non-toxic. On the other hand, the retronecine-, heliotridine-, and otonecine-type pyrrolizidine alkaloids that have a double bond at the C1 and C2 positions of the necine base exhibit high levels of toxicity, including hepatotoxicity and carcinogenicity. Consequently the most attention has been focused for these three types of pyrrolizidine alkaloids[2,5,7,8,12,16,17].

METABOLIC ACTIVATION OF PYRROLIZIDINE ALKALOIDS

Since pyrrolizidine alkaloids require metabolic activation in order to exert genotoxicities, metabolism study of pyrrolizidine alkaloids has been extensively conducted[7,12,16,73-99]. The majority of the retronecine-, heliotridine-, and otonecine-type pyrrolizidine alkaloids are highly toxic and many are tumorigenic (Figure 2), and consequently, metabolism of pyrrolizidine alkaloids of these three types has been studied to the greatest extent. The retronecine-type and heliotridine-type pyrrolizidine alkaloids, which are enantiomers at the C7 position, exhibit three principal metabolism pathways, namely; (i) hydrolysis of the ester functional groups to form the necine bases and acidic metabolites; (ii) *N*-oxidation of the necine bases to the corresponding *N*-oxides; and (iii) formation of the corresponding dehydropyrrolizidine (pyrrolic) derivatives through hydroxylation at the C-3 or C-8 position of the necine base to form 3- or 8-hydroxynecine derivatives followed by dehydration[92].

Recent studies by Lin and co-workers on an representative otonecine-type pyrrolizidine alkaloid, clivorine, established that metabolism of otonecine-type pyrrolizidine alkaloids leads to metabolites similar to those from metabolism of retronecine-type and heliotridine-type pyrrolizidine alkaloids[89,90,92]. The principal metabolic pathways involve (i) hydrolysis of the ester functional groups to form the corresponding necine bases and acids and (ii) formation of the corresponding dehydropyrrolizidine (pyrrolic) derivatives through oxidative N-demethylation of the necine base followed by ring closure and dehydration.

While hydrolysis and N-oxidation are considered as detoxification pathways, formation of dehydropyrrolizidine (pyrrolic) metabolites is generally recognized as the metabolic activation pathway responsible for the genotoxic and tumorigenic activities of pyrrolizidine alkaloids, since these pyrrolic metabolites are chemically reactive. This is evidenced by the fact that once formed dehydropyrrolizidine (pyrrolic) compounds can rapidly bind with DNA leading to DNA cross-linking, DNA-protein cross-linking, and DNA adduct formation[7,9,12,61,62,93,100]. However, pyrrolic metabolites can also react with water and other cellular constituents to form the detoxified products. It has been demonstrated that pyrrolic metabolites can bind with glutathione to produce glutathione conjugates. This enzymatic reaction, catalyzed by glutathione S-transferase, is possibly a major detoxification pathway[89,90,101-103]. Lin and co-workers studied rat liver microsomal metabolism of clivorine[89,90] and identified dehydroclivorine (the pyrrolic ester) as a reactive metabolite that covalently bound to the tissue constituents in the liver and induced liver damage. This metabolite (dehhydroclivorine) can further react with glutathione to form four glutathione conjugates and this biotransformation is considered as a detoxification process (Figure 5). Thus, factors that may either increase the metabolic rates for the activation of clivorine to generate the reactive pyrrolic ester and for the formation of the toxic tissue-bound pyrroles, or decrease the metabolic rates for detoxification glutathione conjugations may play the key roles in clivorine genotoxicity[90].

Metabolism of pyrrolizidine alkaloids to form dehy-

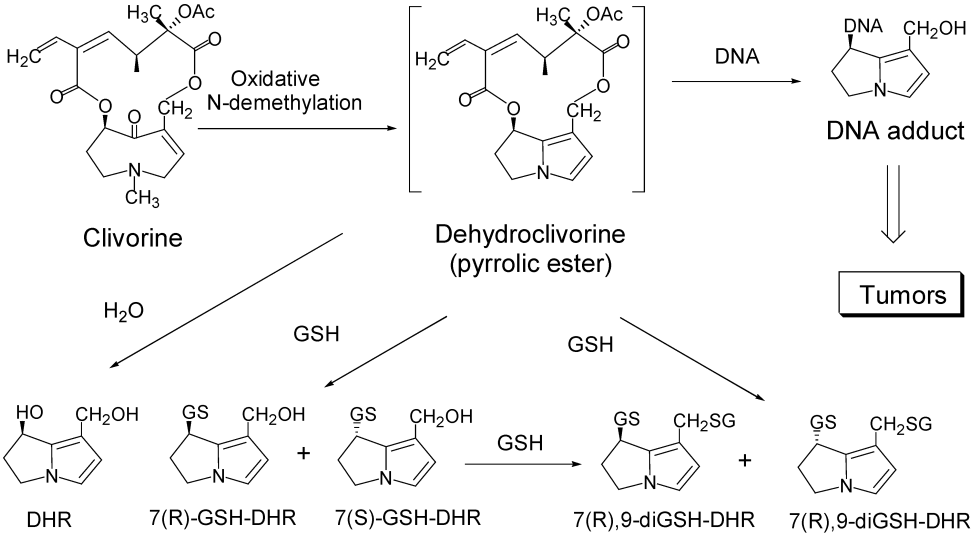

Figure 5. Metabolic detoxification and activation of clivorine.

dropyrrolizidines, the reactive pyrrolic ester, is mainly catalyzed by cytochrome P-450 monooxygenases, specifically the CYP3A and CPY2B6 isoforms[75-82,90,93]. Metabolism of pyrrolizidine alkaloids to produce the corresponding pyrrolizidine alkaloid N-oxides is catalyzed by both cytochrome P-450 and flavin-containing monooxygenases[77,81,105,106]. Furthermore, hydrolysis of the ester functional groups to generate the corresponding necine base and acidic metabolites is mainly mediated by liver microsomal carboxylesterases[75-82,93].

It has been reported that the species difference in susceptibility to the pyrrolizidine alkaloid toxicities is mainly due to the variations in the balance between the formation of the toxic pyrrolic metabolites and the detoxification pathways to generate hydrolyzed metabolites and/or nontoxic N-oxides[7,104]. The species difference in susceptibility to the otonecine-type pyrrolizidine alkaloid induced hepatotoxicity was studied by Lin *et al.*[92]. The *in vitro* metabolic activation of clivorine in both male rat and human was similar but was different from that in guinea pig. The higher activation rates for the generation of the reactive pyrrolic ester followed by the formation of the toxic tissue-bound pyrroles mainly contribute to the high susceptibility of human and male rat to clivorine hepatotoxicity. The higher metabolic rates for the hydrolysis in combination with a lower rate for the formation of toxic tissue-bound pyrroles play a key role in guinea pig resistance to clivorine intoxication.

MECHANISMS LEADING TO TUMORIGENICITY

There are several mechanistic activation pathways by which pyrrolizidine alkaloids exert their tumorigenicity. Pyrrolizidine alkaloids are mutagenic in Salmonella, suggesting that they may induce tumors through a genotoxic mechanism mediated by pyrrolizidine alkaloid-derived DNA adduct formation. Several tumorigenic pyrrolizidine alkaloids have been shown to cause DNA cross-linking and DNA-protein cross-linking in cultured epithelial cells. Thus, formation of DNA cross-linking and/or DNA-protein cross-linking may lead to tumor formation; although further investigation is warranted for confirmation. Since pyrrolizidine alkaloids cause DNA cross-linking and micronucleated polychromatic erythrocytes, they may be clastogens[107]. Pyrrolizidine alkaloids may also induce tumors through lipid peroxidation because administration of antioxidants, such as butylated hydroxyanisole, efficiently reduced the toxic effects of monocrotaline[88,108] and because trans-4 hydroxy-2-hexenal was found as a metabolite of senecionine[64,87].

We have determined that riddelliine is metabolized by male and female human liver microsomes to form 6,7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine (DHP), which leads to the formation of a set of eight DHP-derived DNA adducts[12,93]. The levels of the DNA adduct formation correlated closely with the tumorigenic potencies of the mice fed with different doses of riddelliine[93,98,99]. We have also determined that the metabolism pattern and DNA adduct profiles from human liver microsomes are quite similar to those formed in rat liver *in vitro* and *in vivo* (unpublished data). The kinetic parameters, Vmax and Km, from human liver microsomal metabolism are also comparable to those from rat liver microsomal metabolism. The metabolic study in the presence of a human CYP3A4 inhibitor strongly suggests that the formation of DHP and riddelliine N-oxide from metabolism of riddelliine is principally catalyzed by the CYP3A4 isozyme. Taken together, these results strongly indicate that our previous *in vivo* and *in vitro* mechanistic studies with experimental rodents[93] are highly relevant to humans. Since riddelliine induces liver tumors in male and female rats and male mice[99] and the DHP-derived DNA adducts are responsible for liver tumor induction, these results suggest that riddelliine can be highly genotoxic to humans and the genotoxic mechanism is mediated by DHP-derived DNA adduct formation.

Although a number of pyrrolizidine alkaloids have been found to induce tumors in experimental animals (Table 2), the mechanisms of these compounds leading to tumorigenicity are not clear. Our study on riddelliine provides the first established genotoxic mechanism by which a pyrrolizidine alkaloid (riddelliine) induces liver tumors. We anticipate that this activation pathway may be general to all the retronecine-, heliotridine-, and otonecine-type pyrrolizidine alkaloids and the eight DHP-derived DNA adducts may be formed *in vivo* and *in vitro*. The proposed metabolic activation of clivorine, an otonecine-type pyrrolizidine alkaloid, leading to DNA adduct formation and tumor formation is shown in Figure 5.

IDENTIFICATION AND QUANTITATION OF TOXIC PYRROLIZIDINE ALKALOIDS IN CHINESE HERBAL PLANTS AND DIETARY SUPPLEMENTS

For human health protection, it is important to insure that the use of herbal medicines (as drugs), herbal teas (as food), and dietary supplements (as health food) is safe. The widespread distribution of toxic pyrrolizidine alkaloids in the environment can cause serious food contamination leading to livestock poisoning, and eternally resulting in human health damage. Consequently, in Europe, United States, and Australia, simple, convenient and accurate analytical methods have been developed for characterization and quantification of toxic pyrrolizidine alkaloids present in herbal plants, dietary supplements, food products, and poisoned animals[7,15,17,29,39,40,42,109-120]. These methods include the use of: (i) a quantitative spectrophotometric method (the Ehrlich reagent) and trapping strategies for quantifying the reactive pyrrolic metabolites *in vivo* and *in vitro*; (ii) solid-phase extraction and HPLC-MS, GC-MS, and HPLC-UV analysis for identification and quantification of the pyrrolizidine alkaloids in the plants; and (iii) competitive inhibition enzyme-linked immunosorbent assays and mass

Journal of Food and Drug Analysis, Vol. 10, No. 4, 2002

spectrometry for determining the amount of pyrrolizidine alkaloids in biological samples. For example, the Ehrlich reagent photometric method, originally developed by Mattocks in 1967, has been popularly used to detect pyrrolizidine alkaloids and pyrrolizidine alkaloids *N*-oxides contained in the plants[110]. This method was recently modified by Roeder's group to detect otonecine-type pyrrolizidine alkaloids, including senkirkine, a reference pyrrolizidine alkaloid used for the gas chromatographic determination of pyrrolizidine alkaloids in plants and other sources[117]. This method is highly sensitive, to be able to detect 1 ppm of pyrrolizidine alkaloids present in plants; however a drawback is that the identity of the pyrrolizidine alkaloids can not be determined. Consequently, for structural identification and quantification, on-line GC-MS and HPLC-MS are the most convenient analytical methods[7,15,42,91,112,113,115]. The on-line GC-MS method has also been the most effective methodology for detecting pyrrolizidine alkaloids contaminating the food chain, such as honey and milk[7,15,17], although the other analytical methods, including thin-layer chromatography (TLC) and HPLC, are also employed. Competitive inhibition enzyme-linked immunosorbent assays have also been developed for detection of poisoned livestock[119]. These simple, rapid, and sensitive methods can accurately detect pyrrolizidine alkaloids and pyrrolizidine alkaloid N-oxides in biological samples.

REGULATORY CONCERN ON USING PYRROLIZIDINE ALKALOID-CONTAINING HERBAL PLANTS AND DIETARY SUPPLEMENTS

Because pyrrolizidine alkaloids present in staple food and herbal medicines can result in human poisoning and death, the International Programme on Chemical Safety (IPCS) determined that pyrrolizidine alkaloids present in food are a threat to human health and safety[9,121]. After having established that toxic and tumorigenic pyrrolizidine alkaloids are present in herbal plants, regulatory decisions have been made in several countries. In 1992, the Federal Health Department of Germany has restricted "the manufacture and use of pharmaceuticals containing pyrrolizidine alkaloids with a unsaturated necine skeleton". The herbal plants "may be sold and used only if daily external exposure to no more than 100 µg pyrrolizidine alkaloids and internal exposure to no more than 1 µg per day for no more than six weeks a year"[2,8].

In 1994, the U.S. Congress passed the Dietary Supplement Health and Education Act (DSHEA) that amended the U.S. Federal Food, Drug, and Cosmetic ACT (FFDCA) and created a new regulatory category, safety standard, and other rules for the U.S. Food and Drug Administration (FDA) to regulate dietary supplements. Since then the use of dietary supplements and functional foods has grown quite rapidly. It was reported that in 1999, U.S. consumed about US$31 billion for dietary supplements and functional foods. Expense for functional foods is projected up to US$49 billion by 2010. Under such a circumstance, it is important to ensure their therapeutic efficacy and health safety. Any herbal plants containing toxic components, such as the hepatotoxic and tumorigenic pyrrolizidine alkaloids, should be avoided for use or used under well-controlled conditions. To ensure quality control on dietary supplement products, in 1997 the U.S FDA published Good Manufacturing Practice (GMP) regulations that manufactures of herbal products must follow.

PERSPECTIVES

I. *Identification and Quantification of Toxic Pyrrolizidine Alkaloids in Chinese Herbal Plants*

China is the origin of using TCM for clinical practice. A variety of Chinese herbal plants are produced in tremendous amounts from China annually for domestic use as well as for exporting to foreign countries. Many of these herbs are also used to prepare functional foods, dietary supplements, and herbal teas sold to many countries in the world. Therefore, besides for therapeutic efficacy, quality control and quality assurance of Chinese herbal plants are important for human health protection. The recent efforts on pursuing quality control and quality assurance of Chinese herbal medicine in China, such as the requirement of Good Manufacture Practice (GMP), have made considerable progress. However, work on toxicity, particularly genotoxicity, requires further improvement. Since pyrrolizidine alkaloids are toxic, it is important to determine whether or not there are still more TCM herbs that contain toxic pyrrolizidine alkaloids. As such, it is important to know - Are there many Chinese herbal plants that contain toxic pyrrolizidine alkaloids have not been identified? It is hard to answer since the majority of the Chinese herbal plants grown in China have not been well examined. Therefore, it is important to identify TCM herbs that contain toxic pyrrolizidine alkaloids and make any necessary regulatory decision on the use of pyrrolizidine alkaloid-containing TCM herbs in China.

II. *Toxicity of Pyrrolizidine Alkaloid N-oxides - A safety Consideration*

It has been determined that the quantities of pyrrolizidine alkaloids and pyrrolizidine alkaloid N-oxides present in plants are nearly equal, although their relative amounts in a plant varies. The quantities of pyrrolizidine alkaloid *N*-oxides in many cases are much greater than the parent pyrrolizidine alkaloids and in some cases may be exclusively the *N*-oxide form[7]. In most cases, prior to solvent extraction of pyrrolizidine alkaloids from the plants for biological and toxicological studies, pyrrolizidine alkaloid N-oxides are chemically reduced into the parent pyrrolizidine alkaloids. Consequently, the adverse biological properties of pyrrolizidine alkaloid N-oxides have been much less studied. Of particular importance is that

pyrrolizidine alkaloid N-oxides are more water soluble than the corresponding pyrrolizidine alkaloids. When plants containing pyrrolizidine alkaloids and pyrrolizidine alkaloid N-oxides are used as herbal tea or herbal medicine, such as Chinese herbal medicine, much more pyrrolizidine alkaloid N-oxides will be simmered (extracted) out than the pyrrolizidine alkaloids and drunken by the patients. Consequently, it is important to assess the risk to humans posed by drinking TCM herb-derived decoctions that contain pyrrolizidine alkaloids and/or pyrrolizidine alkaloid N-oxides.

III. *Determination of Mechanism of Actions*

The mechanisms by which pyrrolizidine alkaloids exert genotoxicity and tumorigenicity are not well understood and warranted for further investigation. Assessment of human health risk posed by exposure to pyrrolizidine alkaloids has to be justified based on mechanism. Only those intervention strategies that are based on mechanistic understanding are expected to be the most effective.

IV. *Intervention of Pyrrolizidine Alkaloid Induced Genotoxicity*

It is relevant to study intervention strategies for prevention of human health risk posed by pyrrolizidine alkaloids. Following the principle of TCM, herbal doctors prepare a formula consisting of a number of herbs for treatment for patients after diagnosis. The formula in general contains one major active herb and several supporting herbs to minimize its adverse toxicological effects and/or enhance the drug efficacy. Consequently, study of interactions among the herbs used in a formula may help find out intervention strategies against toxicity exerted by pyrrolizidine alkaloid present in an herbal plant when this herbal plant is used alone. Recently, Lin *et al.*[122] reported that rats pretreated with glycyrrhizin and glycyrrhetinic acid resulted in the reduction of retrorsine-induced hepatotoxicity. Since both glycyrrhizin and glycyrrhetinic acid are major ingredients of liquorice, a Chinese herbal plant most commonly used in TCM clinical practice, the results of this study support the use of TCM herbal components as well as TCM formula as a useful intervention strategy against pyrrolizidine alkaloid-induced toxicity.

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Pyrrolizidine Alkaloids（吡咯烷士啶生物鹼）—中草藥與膳食補充品之致癌成分

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摘　要

雖然中國及其他亞洲國家長久以來使用傳統中藥治療疾病，且近年來西方國家亦用於新藥開發或供機能食品與膳食補充品使用，然對其品質保證及毒性之研究尚嫌不足。吡咯烷士啶生物鹼（Pyrrolizidine Alkaloids）為肝毒性及致腫瘤性之化合物，某些中草藥及膳食補充品已證實含有該等生物鹼。本文將簡介產於中國大陸含此類生物鹼之中草藥，以及此類化合物之毒性、基因毒性與致腫瘤性。文中亦將介紹其代謝途徑，尤其導致基因毒性之活化途徑。最近其致毒機制研究顯示吡咯烷士啶生物鹼（Pyrrolizidine Alkaloids）之致腫瘤性，係經由形成6,7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine (DHP) 所衍生之DNA加成物導致基因毒性機制。大部分吡咯烷士啶生物鹼（Pyrrolizidine Alkaloids）其致癌性屬此類機制，本文並將對未來研究方向提供建議。

關鍵詞：Pyrrolizidine alkaloids; Chinese herbal medicines; tumorigenecity