23 ADME of Natural Toxins

VANESSA GONZÁLEZ-PÉREZ

Department of Pharmacotherapy and Experimental Therapeutics, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

DAVID J. KROLL

Genomics and Microbiology Research Laboratory, Nature Research Center, North Carolina Museum of Natural Sciences, Raleigh, NC, USA

23.1 INTRODUCTION

23.1.1 What are Natural Toxins?

A common misperception among the public is that any product derived from nature is inherently safe. Nothing could be farther from the truth. Some of the compounds that are most toxic to humans and other mammals occur in nature. History is rife with stories of murder and poisonings from natural toxins, from the death of Socrates via ingestion of poison hemlock (coniine alkaloids from *Conium maculatum*) in 399 BC to the 1978 murder of Bulgarian writer Georgi Markov with an umbrella outfitted with a ricin-filled pellet.

For the sake of this chapter, natural toxins are defined as potentially harmful chemicals produced by living organisms, such as plants, animals, fungi, and algae, or those found naturally occurring in the environment, such as toxic metals. Of course, the

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Paracelsan tenet notes that all substances are potentially poisonous depending on the dose. Here, we will consider representative, small-molecule compounds most commonly associated with severe adverse reactions and death.

The production of natural toxins by living organisms is most often the result of secondary metabolism of natural products. Natural toxins may be ingested, inhaled, or absorbed through the skin. Of greatest concern is that natural toxins may enter the food supply at many different points and may be ingested by animals or humans, therefore representing a threat to human health. Increasing attention is also being given to toxic mold contamination of buildings and the risks of inhaled natural toxins.

Over decades, there have been reports on the acute, chronic, and late effects of exposure to natural toxins in both isolated communities and across large populations. Moreover, there have been correlations of their consumption and disposition with late effects of exposure, such as increased risk of cancer from aflatoxins [1]. As a result, there has been much interest in understanding the mechanisms of absorption, distribution, metabolism, and excretion of these compounds and minimizing exposure.

Given the relevance of natural toxins in human health, much attention has been given to identify their sources and understand their effects on the human body. Organizations such as the World Health Organization (WHO), International Program on Chemical Safety (IPCS), US Food and Drug Administration (FDA), and the American Association of Poison Control Centers will commonly issue warnings on toxicology and poisoning trends as well as emergency management of adverse effects of natural toxins.

In the following chapter, we discuss some examples of natural toxins, their sources, and notable mechanisms of metabolism and excretion that influence the toxicity of these chemicals.

23.2 AFLATOXINS

Aflatoxins are the most studied of all fungal toxins or mycotoxins. Aflatoxins are produced primarily by two fungal species, *Aspergillus flavus* and *Aspergillus parasiticus.* These organisms are common saprophytes and opportunistic pathogens with high occurrence in tropics or semitropic regions [2]. Aflatoxins are by-products of fungal metabolism with the major types of metabolites aflatoxin B_1 (AFB_1), aflatoxin B_2, aflatoxin G_1, aflatoxin G_2, and aflatoxin M_1 [2]. Aflatoxins are detected occasionally in milk, cheese, corn, peanuts, cottonseed, Brazil nuts, walnuts, pistachios, pecans, almonds, figs, spices, and a variety of other foods and feeds. Milk, eggs, and meat products are sometimes contaminated because of animal consumption of aflatoxin-contaminated feed. However, the materials with the highest risk of aflatoxin contamination are corn; cereals such as rice, groundnuts, and peanuts; and cottonseed [2].

Factors contributing to the presence or production of mycotoxins in foods or feeds include storage, environmental, and ecological conditions, particularly high humidity environments that encourage growth of *Aspergillus* species normally found in soil [3]. Mycotoxicoses in humans or animals are characterized as food- or feed- related, noncontagious, nontransferable, noninfectious, and nontraceable to microorganism other than fungi [3].

Aflatoxins were originally identified in 1960 as the cause of the death of thousands of turkeys due to hepatic necrosis [4]; their feed was identified as the source of the toxin. To date, the largest case of aflatoxicoses reported in humans occurred in western India in 1974, resulting in 397 recognized cases and 106 deaths [5]. However, additional outbreaks have been reported throughout the world, some examples include Eastern and Central provinces of Kenya in 1981 and most recently, in 2004, where the outbreak resulted from widespread aflatoxin contamination of locally grown maize [6]. The incidence of aflatoxin-driven outbreaks often generates concern about the safety of those exposed and the rate at which neighboring developments show the symptoms. For every symptomatic case of aflatoxicosis identified, several other persons likely were exposed and might face future adverse health consequences. Thus, there is a need to execute routine food inspections to ensure food safety and local education and assistance to ensure that foods such as maize are harvested correctly, dried completely, and stored properly.

Aflatoxin exposure can result in acute aflatoxicosis (poisoning caused by ingestion of high levels of aflatoxin) often manifested as severe, acute hepatotoxicity. Among the early symptoms of hepatotoxicity include anorexia, malaise, and low grade fever. After the acute exposure, the symptoms progress to lethal hepatitis with vomiting, abdominal pain, jaundice, fulminant hepatic failure, and death [7]. In contrast, chronic aflatoxicosis (poisoning caused by chronic ingestion of low to moderate levels of aflatoxin) typically presents with impaired food conversion and slow growth. In addition to aflatoxicosis, aflatoxin exposure can lead to carcinogenesis, particularly hepatocellular carcinoma [1,8,9] as recently reviewed in Ref. 10).

A somewhat controversial approach to prevent aflatoxin exposure via corn and peanuts has been undertaken by the international agribusiness firm Syngenta with the introduction of their Alfa-Guard product. Afla-Guard is a hulled barley delivery system for spores of a non-aflatoxin-producing strain of *A. flavus* that actively competes for growth by toxic *A. flavus*. The product originated in the US Department of Agriculture's Agricultural Research Service with the work of microbiologist Joe Dorner at the National Peanut Research Laboratory in Dawson, GA.

Despite all of our knowledge on the symptoms and detection of aflatoxin poisoning, it is likely aflatoxins will continue to be a public health problem until appropriate storage methods for food are implemented worldwide. But even in areas of the world with ideal crop storage conditions, *A. flavus* may be unavoidable. The most critical public health approach is continued monitoring of food products for aflatoxins and vigilant surveillance and detection of aflatoxin exposure.

23.2.1 ADME of Aflatoxins

The primary route of absorption of aflatoxins is via ingestion of contaminated food. However, aflatoxins can be found in the dust of contaminated grains and inhalation has been associated with increased risk of lung cancers [11].

Aflatoxins are a classic example of natural toxins that must undergo metabolism to exert their untoward effects. In fact, this theme is common among microbial and plant-based xenobiotics that are toxic to mammals [12].

Aflatoxin activation is mediated via cytochrome P450s (CYPs). Initial debate focused on whether CYP3A4 is the dominant enzyme for AFB_1 metabolism [13,14] relative to the contribution of CYP1A2 [15]. A later study from Gallagher and

colleagues suggested that CYP3A enzyme(s) are capable of AFB_1 oxidation at relatively high substrate concentrations; however, CYP1A2 appears to be the high affinity isoform that is active at lower substrate concentrations when examined in human liver microsomes [16,17]. However, a more recent study on AFB_1 metabolism indicates a predominant role of CYP3A4 for AFB_1 disposition in human liver [18]. In lung cells transfected with cDNA expression constructs for CYP1A2 or CYP3A4, both were capable of AFB_1 activation at low substrate concentrations [11].

Among AFB_1 biotransformation products, activation to the reactive aflatoxin B_1-8,9-epoxide (AFBO) is necessary to exert its hepatocarcinogenic effects, since AFBO binding to cellular DNA, is highly correlated to the carcinogenic potency of AFB_1 [16]. Studies of aflatoxin metabolites have demonstrated covalent binding of AFB_1 to DNA as the AFB_1-N^7-guanine adduct, 2,3-dihydro-2-(N^7-guanyl)-3-hydroxy AFB_1 [19]. These adducts can cause G > T transversions and other mutations that lead to tumor formation [20].

In addition to DNA adduct formation, AFBO can also bind other proteins and critical cellular nucleophiles [10]. An example of AFB_1 binding proteins is albumin, the major transporter of AFB_1 in blood [21]. Serum AFB–albumin adducts, are associated positively with hepatocellular carcinoma in humans [22], and analysis of serum adducts has also indicated a positive correlation between dietary AFB_1 exposure and serum AFB_1–albumin adducts [23].

Another example of a toxic adducts is the interaction of AFB_1 with the signal recognition particle (SRP), which acts as an escort for polyribosomes with signal peptides to be transported and bound to the cytoplasmic face of the endoplasmic reticulum (ER) during protein targeting [24]. The interaction of AFB_1 with SRP is now known to generate structural alterations to prevent biosynthesis and translocation of secretory proteins that are important components of the plasma membrane, gap junctions, and intercellular matrix. Singh *et al*. [25] hypothesize that these interactions could disturb cell-to-cell communication and contribute to tumorigenesis.

Aflatoxins are metabolized in liver to oxidized derivatives, including AFBO and AFM1, that are secreted in the bile and excreted in urine, with a small proportion (about 1% of the ingested dose) being activated through the epoxide to form covalent adducts with DNA [8]. In humans and most animals, the principal route of AFB_1 detoxification is through conjugation with endogenous glutathione (GSH), a reaction catalyzed by glutathione-*S*-transferases (GST) [10]. Circumstantial evidence is suggestive that GST disposition of aflatoxin metabolites plays a major role in determining the sensitivity of cells to AFB_1 [26]. Essigmann *et al*. [27] first evaluated the possibility of detecting DNA adducts caused by AFB_1 ingestion in human urine, with great success. Since then, detection of DNA adducts or AFBO–albumin complexes in the urine have been a reliable marker for the presence of aflatoxin metabolites.

23.3 ARISTOLOCHIC ACID

Aristolochic acid (AA) is a chemical found in the extract of the plant *Aristolochia fangchi* [28]. Usually, AA is a mixture of structurally related nitrophenanthrene carboxylic acids, with 8-methoxy-6-nitro-phenanthro-(3,4-*d*)-1,3-dioxolo-5-carboxylic acid I known as *aristolochic acid I* (AAI) and the demethoxy analog also known as *aristolochic acid II* (AAII) [29]. For many years, AA was utilized for a variety of

treatments summarized elsewhere [29,30] until it was found that the use of herbal drugs containing AA can cause permanent kidney damage, including end-stage kidney failure, requiring dialysis or kidney transplantation, and cancer associated with the urinary tract.

As a result, in 2001, the FDA released a list of compounds containing AA (http://www.fda.gov/Food/DietarySupplements/Alerts/ucm095297.htm) [31]. Moreover, despite the long use of AA in herbal remedies for the treatment of numerous symptoms [29], it was not until the early 1990s when cases of advanced renal failure were reported in Belgium, where it was reported in 1991 that consumption of AA in herbal preparations inadvertently included in slimming pills had a correlation with Chinese herb nephropathy (CHN), a type of progressive renal fibrosis [32,33]. In addition to this incident, AA-driven DNA adducts have also been correlated with Balkan endemic nephropathy patients [34]. Once the role of AA was identified as the cause of CHN, the disorder was then proposed to be referred to as AA nephropathy [35].

In addition to the nephrotoxic effects of AA, correlation with the effects of this toxin in carcinogenesis and mutagenesis [36,37], and with the formation of AA DNA adducts [38], has also been demonstrated. More specifically, the activation of AA to AAI has led to various reports indicating that the AAI-driven DNA adducts are the main cause of malignant transformation [29,30] by inserting an A > T mutagenic transversions affecting adenines of codon 61 of the H-Ras mouse gene [37] or purines in the human tumor suppressor p53 gene [39]. Grollman *et al*. [40] further confirmed the presence of artistolactam-dA and artistolactam-dG lesions in the p53 genes in patients with Balkan endemic nephropathy (EN), defined in this chapter as a chronic tubulointerstitial disease frequently associated with urothelial carcinomas of the upper urinary tract. The authors cite the source of the exposure as *Aristolochia clematitis*, a plant endemic to wheat fields of the region. Consumption of baked breads made from grains contaminated with low levels of *A. clematitis* is believed to be responsible for this chronic disease.

23.3.1 ADME of Aristolochic Acid

AA is primarily absorbed in the body via oral ingestion, either via contaminated grain or in weight-loss diet pills containing extracts of *A. fangchi* [41]. Given that AA is predominantly metabolized by hepatic CYP1A2, the kidney and urinary tract are the predominant sites of toxicity [30]. More specifically, there is also evidence that epithelial cells of the proximal tubule are the primary cellular target of AA [42]. Therefore, the liver, kidney, and transitional uroepithelial cells are the main tissues in which AA metabolites are distributed.

In vitro studies, under anaerobic conditions, have suggested that the most important human enzymes activating AA to AAI are hepatic microsomal CYP1A2, renal microsomal NADPH:CYP reductase [43], hepatic and renal cytosolic NAD(P)H:quinone oxidorectudase (NQO1), as well as cyclooxygenase (COX), which is highly expressed in the urothelial tissue [28]. Of all the activating enzymes mentioned above, one of the most efficient AA activating enzymes is NQO1, ubiquitously present in all tissue types [30].

On the other hand, there is also evidence that under aerobic conditions, hepatic microsomes from rats and humans demethylate AAI to form AAIa [44]. AAIa (8-hydroxy-6-nitro-phenanthro-(3,4-*d*)-1,3-di-oxolo-5-carboxylic acid) is the nontoxic

derivative of AA. *In vivo* studies by Xiao and colleagues [45] suggested that hepatic CYP1A enzymes, specifically CYP1A2, also play a critical role in detoxification and prevention of AAI-induced kidney injury. Therefore, depending on the systematic conditions, CYP enzymes can play the role of AA activator or AAI excretion.

AA metabolites are excreted in urine. As described above, the main effects of AAI in the human body is the formation of DNA adducts in animals and humans. As a result, AA–DNA adducts have been validated and used as biomarkers of exposure to AA and to investigate the mutagenic and carcinogenic potential of AA. The major AA–DNA adducts found in rodents exposed to AA and in patients suffering from AAN were identified as 7-(deoxyadenosin-N^6-yl) aristolactam I (dA-AAI), 7-(deoxyguanosin-N^2-yl) aristolactam I (dG-AAI), and 7-(deoxyadenosin-N^6-yl) aristolactam II (dA-AAII) [30,46].

23.4 ARSENIC

Arsenic (As) is a highly poisonous metallic element. Inorganic As occurs naturally in the environment and exposure can occur via leaching from geological formations and occupational exposure such as in mining activities, metal processing, and application of pesticides [47].

As exposure can occur from food, air, and water, but the major chronic As exposure comes from the most precious and needed element for survival, water. Among the arsenic species identified in natural water are inorganic arsenite (As^{III}), arsenate (As^{V}), monomethylarsonic acid (MMA^{V}), monomethylarsonous acid(MMA^{III}), dimethylarsinic acid (DMA^{V}), and dimethylarsinous acid (DMA^{III}); As^{III} and As^{V} are by far the most toxic to humans [48]. Therefore, it is vital to regulate the levels of As in water sources all around the world as a method to control As poisoning (arsenicosis) throughout the world. As exposure is correlated with several types of cancer such as the skin, bladder, and lung, reviewed in detail by Kapaj and colleagues [47] and others [49]. In addition to cancer, As also has effects in memory and intellectual functions, cardiovascular disease, respiratory system diseases, and diabetes among others [47].

The mechanisms by which As causes carcinogenicity is by exerting either genotoxic effects (e.g., chromosomal abnormalities, oxidative stress, and gene amplification) or nongenotoxic effects (e.g., altered growth factors, enhanced cell proliferation, and altered DNA repair) as reviewed in detail elsewhere [50].

23.4.1 ADME of Arsenic

As can be absorbed through either the respiratory or the digestive system. Human inhalation exposure to inorganic arsenic can occur through occupational exposure (e.g., coal-fired power plants) or during cigarette smoking [51]. The process starts when As particles are first deposited in the lungs, where it is later absorbed into the bloodstream, depending on the solubility of the chemical form of As. Evidence of As absorption can be detected in the urine, making possible to determine the As exposure in humans as reported by Offergelt *et al*. [52], Yager *et al*. [53], and others.

After the absorption of As through either the respiratory or digestive system, As metabolites enter the bloodstream, to be distributed to the different parts of the body. Exposure to As leads to an accumulation of As in tissues such as skin, hair, and nails,

resulting in various clinical symptoms such as hyperpigmentation and keratosis [47]. The analysis of Scottish and Japanese individuals after long-term chronic exposure showed that As can also accumulate in bone, teeth, muscle, brain, and heart among other organs as summarized in Ref. 48.

In humans, As is excreted as both methyl arsenic (MAs) and dimethyl As (DMA) being the second the main metabolite excreted by the body and detected in the urine of exposed patients [54,55]. The metabolism of arsenic is characterized in many species by two types of reactions: (1) reduction of pentavalent to trivalent arsenic and (2) oxidative methylation reactions in which trivalent forms of arsenic are sequentially methylated to form mono-, di-, and trimethylated products using *S*-adenosyl methionine (SAM) as the methyl donor and GSH as an essential cofactor [51].

Each oxidative methylation reaction is preceded by reduction of arsenic from the pentavalent to the trivalent oxidation state. These reactions are catalyzed by a cytosolic enzyme, arsenic (+3 oxidation state) methyltransferase (As3mt), which uses *S*-adenosylmethionine (AdoMet) as a methyl group donor and requires a dithiol-containing reductant (e.g., thioredoxin) for catalysis [56,57].

Urinary excretion of arsenic begins when arsenic metabolites in the blood are removed by the kidneys about 5 h after ingestion [54]. Early reviews in the field have reported that the arsenic metabolites entering the bloodstream are excreted in several forms, including arsenite (As+3), arsenate (As+5), methyl dimethylarsinic acid (DMAA), and other organically bound arsenic compounds [54]. As can also be excreted through the skin, nails, hair, and sweat but to a far lesser quantitative extent.

While arsenic is considered toxic during acute or chronic oral or inhalation exposure, intravenous arsenic trioxide (As_2O_3) was investigated in China during the early 1990s as a treatment for acute promyelocytic leukemia (APL) [58]. The same research team that had popularized the use of all-*trans*-retinoic acid for APL had been investigating traditional Chinese remedies and identified an antileukemia component as arsenic trioxide [59]. The anticancer effects of arsenic trioxide appear to be due in part to inducing BH-3 family proapoptotic mediators [60]. Second-generation organic arsenicals may continue to have efficacy in cancers that have become resistant to arsenic trioxide [61].

23.5 SELENIUM

Of all the natural products to which humans are exposed, not all can be classified as either beneficial or toxic. This is the case for the trace element Selenium (Se), which can be both beneficial or toxic depending on the exposure and/or doses administered. Se is an essential trace element for humans, animals, and some bacteria [62].

Se is considered essential due to its association with proteins, known as *selenoproteins* [63]. Selenoproteins have biological functions in oxidoreductions, redox signaling, antioxidant defense, thyroid hormone metabolism, and immune responses [64]. Given the important role of selenoproteins in physiological functions, selenoproteins possess a strong correlation with human diseases such as cancer, Keshan disease, virus infections, male infertility, and abnormalities in immune responses and thyroid hormone function [64].

While an essential nutrient, Se can also be toxic depending on the exposure levels. For example, Se compounds at low concentration may have protective anticarcinogenic

properties, whereas at higher concentration, they can be genotoxic and possibly carcinogenic [65]. Chronic toxicity of selenium in humans results in a condition termed *selenosis,* characterized by hair and nail loss and brittleness, gastrointestinal problems, skin rash, garlic breath odor, and nervous system abnormalities [66]. Other related toxic effects caused by Se are disruption of endocrine function, including synthesis of thyroid hormones and growth hormones, and insulin-like growth factor metabolism [67]. The mechanism of Se toxicity has not been clarified but is mostly attributed to its ability to induce oxidative stress both *in vitro* and *in vivo* [62,68].

Se exists mostly in organic forms in normal diets. Organic Se is present in foods mainly in the form of selenomethionine, selenocysteine, and Se methylselenocysteine, whereas inorganic Se either as selenite or as selenate occurs much less frequently and in very low amounts [69]. Of the organic forms, selenomethionine is the predominant form in most Se-rich diets. Both organic and inorganic forms of Se appear to be utilized with similar efficacy in the body to produce selenoproteins [70]. Given that Se is a nutrient whose deficiency and toxic concentrations are very close to each other, it is important to know about its abundance or deficiency in food and diet to establish the right balance of Se in humans and animals [67] and to establish clear parameters of the maximum tolerated doses to benefit from it and also prevent its toxic effects.

23.5.1 ADME of Selenium

The main source of Se is food, and the content of Se can vary much depending on the geographical location, seasonal changes, protein content, and food processing [67]. Se *exposure* occurs mostly through ingestion of Se-containing foods, with much of dietary Se coming from nuts, cereals, meat, fish, and eggs. The levels of Se in different foods and beverages vary and have been reviewed in detail in Ref. 67. Se bioavailability strongly depends on the chemical form of Se found in food, and generally, organic forms of Se are more bioavailable than the inorganic forms [67,71]. Approximately 80% of dietary Se is absorbed, with wheat and meats the most important Se dietary sources [67]. The absorption of Se occurs in the small intestine as evaluated in many animal models by numerous researchers in the field.

Following Se absorption, it is then distributed throughout the body were Se can be located in the skeletal muscles, although organs such as the kidneys, testes, and liver have the highest relative concentration of Se. In contrast, cells that reveal a higher consumption of Se are those of the immune system, erythrocytes, and platelets [67].

Inorganic forms of selenium (selenite and selenate) are reduced by GSH to generate hydrogen selenide (H_2Se) intermediates that later become incorporated into selenoproteins. Organic forms of Se can also become the H_2Se intermediate, where H_2Se is the intermediate compound between the reductive metabolism of Se and its methylation pathway [69]. Selenium compounds such as selenite, selenodiglutathione, and selenocystine are substrates for *antioxidant enzymes* thioredoxin reductase, thioredoxin, and glutathione peroxidase among others [72]. A by-product from Se metabolism is the formation and accumulation of reactive oxygen species (ROS), which are considered as a source of Se-driven toxicity in the form of DNA damage as reviewed by [69,73,74].

Se is eliminated from the body primarily via the urine, although significant losses via feces also occurs and low amounts of Se are also lost though the skin and respiration [67]. More specifically, there are studies confirming that Se excretion can be rapidly

detected in the urine, where 50–78% of the ingested element is excreted [75], and the levels of secreted Se change depending on the intake of Se in an individuals diet.

23.6 IBOTENIC ACID AND MUSCIMOL

Amanita muscaria is a mushroom with a rich history of use by shamanic cultures of Siberia and northeast Asia. Known also as fly agaric for its use as an insecticide, *A. muscaria* contains the alkaloids ibotenic acid and muscimol and is easily recognizable owing to its red cap with white spots. Saar [76] has described the religious use of *A. muscaria* and English language accounts extending back to the 1700s describe an unusual practice of metabolic significance. A Swedish colonel, Philip Johan von Strahlenberg, held as a prisoner by the Koryak tribe of Siberia wrote in 1730 of his experiences:

> "Those who are rich among them, lay up large provisions of these mushrooms, for the winter. When they make a feast, they pour water upon some of the mushrooms, and boil them. Then they drink the liquor, which intoxicates them. The poorer sort … post themselves, on these occasions, round the huts of the rich, and watch the opportunity of the guests coming down to make water; and then hold a wooden bowl to receive the urine, which they drink off greedily…, and by this way they also get drunk [77]"

The practice, substantiated by George Steller in 1774, owes to the decarboxylation of ibotenic acid to muscimol and a much higher urinary concentration of muscimol than in the mushrooms. Muscimol is an agonist for GABA(A) receptors and produces a lucid dreamlike state that can be perceived as either desirable or disturbing depending on the context. Often mischaracterized as a hallucinogen, muscimol is better described as a deliriant. The receptor specificity of muscimol has led to its use as a radioligand for defining GABA(A) receptors. The parent compound, ibotenic acid, is also a N-methyl-D-aspartate (NMDA) glutamate receptor agonist and is responsible for jerky muscle movements often accompanying accidental ingestion of the mushrooms. In the laboratory setting, ibotenic acid can be used to specifically lesion parts of the brain owing to its excitotoxic effect at NMDA receptors.

Ingestion of *A. muscaria* is rarely fatal. According to the American Association of Poison Control Centers, 61 cases of *A. muscaria* poisonings were reported in 2009.

23.7 α-AMANITIN

While this chapter focuses on small-molecule toxins, the previous discussion of *Amanita* species necessitates some treatment of this classic peptide hepatotoxin. Unlike mushrooms that produce ibotenic acid and muscimol, other species of *Amanita*, most notably not only *Amanita phalloides* but also *Amanita virosa* and *Amanita bisporigera*, are recognized for their production of the cyclic octapeptide, α-amanitin.

Work on the chemistry of toxic "death cap" mushrooms can be traced by to Heinrich Wieland, 1927 Nobel laureate in chemistry for work on the bile salts. Wieland's student at Munich (and later son-in-law), Feodor Lynen, conducted his 1937 PhD dissertation work entitled "On the Toxic Substances in *Amanita*," and Wieland's laboratory later

crystallized the substance in 1941. Lynen went on to investigate the biochemistry and synthesis of cholesterol and fatty acids, work for which he shared the 1964 Nobel Prize in Physiology or Medicine with Konrad Bloch. Work on the amatoxins and related phalloidins was instead undertaken by Heinrich's son Theodor and nephew Ulrich, respectively. While phalloidin exerts toxicity by triggering depolymerization of F-actin, it requires hepatic bioactivation for maximum toxicity. Fluorescent conjugates of phalloidin are used widely today for microscopic imaging of cellular actin networks.

Theodor Wieland worked tirelessly on the chemistry and biochemistry of amatoxins and was the first to separate the α, β, and later γ isoforms by solid-phase electrophoresis [78], a method later used to demonstrate isozymes among enzyme families. The Italian group of Stirpe and Fiume were first to demonstrate in 1967 that α-amanitin rapidly and potently inhibited RNA synthesis [79,80]. Lindell *et al*. [81] later showed in 1970 that the proximal target of α-amanitin was nuclear RNA polymerase II, the RNA polymerase responsible for messenger RNA synthesis. In fact, the selectivity of α-amanitin for the type II polymerase is used to distinguish between the biosynthetic activities of RNA polymerase I (primarily ribosomal RNAs) and III (primarily transfer RNAs).

Symptoms of amatoxin poisoning do not usually present until 6–8 h after ingestion, with severe abdominal cramps, vomiting, and gastrointestinal bleeding that gives rise to hepatic and renal failure [82]. Reports vary based on amount ingested and time of treatment initiation, but most sources cite a 15% fatality rate within 10 days of *A. phalloides* ingestion. Liver transplantation can be curative but is usually not practical. One challenge in antidote management is that α-amanitin undergoes enterohepatic recirculation.

European literature had suggested the potential utility of an intravenous preparation of partially purified extract of milk thistle (*Silybum marianum*), silibinin hemisuccinate (Legalon SIL). This intravenous preparation is produced by Madaus AG and is currently only available in Europe. Two hepatoprotective mechanisms of action are possible: the silibinin compounds may inhibit reuptake of the toxin by intact hepatocytes and/or competitively interfere with glucuronide hydrolysis. Several highly publicized and successful treatment cases in the United States led to an initiation of an open-label clinical trial [83]. The outcome of the ongoing trial is likely to influence the regulatory status of Legalon in the United States.

The reader is referred to an outstanding review in *Science* by Theodor Wieland in 1968 [84] that exhaustively addresses the toxins of all *Amanita* species, from ibotenic acid and muscimol to amatoxins and phalloidotoxins.

23.8 PYRROLIZIDINE ALKALOIDS

Over 350 pyrrolizidine alkaloids can be found in numerous plant species, typically considered noxious weeds, but may also be contaminants of honey, milk, and grains. The most common historical exposure to pyrrolizidine alkaloids has been with herbs purported to have medicinal value. Comfrey, for example, is still sold for making medicinal teas yet is a well-known source of the alkaloids, symphytine, and echimidine [85].

Pyrrolizidine alkaloids are bioactivated by CYP to cause veno-occlusive disease of the liver and cirrhosis after long-term use [11]. This life-threatening disease was first described in 1954 from the ingestion of Jamaican bush tea (*Senecio discolor* and

Crotalaria fulva) but literature reports consistent with this pathology can be found as early as 1920 [86].

CYP3A4 and flavin-containing monooxygenases are responsible for the C- and N-oxidation of pyrrolizidine alkaloids to form the reactive pyrrolic ester and *N*-oxides, respectively.

23.9 GERMANDER (TEUCRIN A)

While pyrrolizidine alkaloids and AA are among the best known plant toxins historically, some localized cases of hepatotoxic herb exposure appear sporadically. In 1991, use of a French weight loss supplement containing wall germander (*Teucrium chamaedrys*) was associated with seven cases of hepatitis that required hospitalization. Pathology ranged from hyperbilirubinemia and jaundice to hepatic necrosis [87]. The hepatotoxicity of two furan-containing diterpenoid components, teucrin A and teuchamaedryn A, is mediated via CYP3A4 oxidation of the 3-substituted furan to an epoxide [88]. The epoxide is proposed to rearrange to form an electrophilic reactive enedial. Druckova *et al*. [89] used teucrin enedial–albumin adducts to create an antibody probe that revealed 46 protein adduct targets in rats administered with teucrin A. The majority of adducted target proteins were found in the mitochondria and ER, such as those involved in lipid metabolism, energy production, cellular respiration, drug metabolism and clearance, and protein chaperones.

While these incidents are infrequent, herbal folkloric use of germander species continues today. A February 2012 case report from Istanbul detailed a case of vomiting and moderate hepatic injury in two-month-old twin sisters who had been given 10 mL of polygermander tea (*Teucrium polium*) added daily to their infant formula for colic [90]. The infants recovered with supportive intravenous fluids and vitamin K administration and were discharged after five days. The continued occurrence of these and other cases of "natural" hepatotoxicity should serve as warning to clinicians to query patients about alternative medicine use on presentation with liver disease of unknown cause [11].

23.10 MACROCYCLIC TRICHOTHECENE (MT) MYCOTOXINS

This class of compounds has become of increasing toxicological importance due to the appreciation of the so-called toxic black mold in human diseases. *Stachybotrys chartarum* and *Stachybotrys chlorohalonata* can colonize water-damaged building materials and cause indoor air exposure to macrocyclic trichothecenes (MTs). These and other species can produce one or more sesquiterpene trichothecenes whose presence can be quantified in indoor air of contaminated buildings. The most common trichothecenes are verrucarins B and J; roridin E; satratoxins F, G, and H; and isosatratoxins F, G, and H.

These compounds are broadly cytotoxic in the nanomolar range and had originally been evaluated by the US National Cancer Institute for potential anticancer activity but their lack of selectivity caused their study to be discontinued. The most noteworthy structural features in their toxicity are the presence of an epoxide moiety (present in all but three MTs) and a diene in the sesquiterpene skeleton. Little work has been done on

the metabolism and excretion of MTs, but it is likely that phase I and phase II enzymes are involved in activation and detoxification of these increasingly relevant toxins.

Work in the yeast *Saccharomyces cerevisiae* indicates that mitochondrial protein synthesis is the target of the trichothecene, trichothecin [91,92]. Several of the compounds are known to cause respiratory symptoms by activating cytokine secretion in human macrophages [93].

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