Piperidine alkaloids: Human and food animal teratogens

Benedict T. Green, Stephen T. Lee, Kip E. Pantera, David R. Brown

1 Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108-6010, USA
2 Poisonous Plant Research Laboratory, Agricultural Research Service, United States Department of Agriculture, USA

A B S T R A C T

Piperidine alkaloids are acutely toxic to adult livestock species and produce musculoskeletal deformities in neonatal animals. These teratogenic effects include multiple congenital contracture (MCC) deformities and cleft palate in cattle, pigs, sheep, and goats. Poisonous plants containing teratogenic piperidine alkaloids include poison hemlock (Conium maculatum), lupine (Lupinus spp.), and tobacco (Nicotiana tabacum) (including wild tree tobacco (Nicotiana glauca)). There is abundant epidemiological evidence in humans that link maternal tobacco use with a high incidence of oral clefting in newborns; this association may be partly attributable to the presence of piperidine alkaloids in tobacco products. In this review, we summarize the evidence for piperidine alkaloids that act as teratogens in livestock, piperidine alkaloid structure–activity relationships and their potential implications for human health.

1. Introduction

Piperidine alkaloids, which possess a characteristic saturated heterocyclic ring structure, occupy a special place in the annals of human and livestock poisoning (Fig. 1). Perhaps the first notable example of poisoning by a member of this alkaloid group was the death of Socrates in 399 B.C. According to the famous account by Plato in Phaedo, the philosopher ingested poison hemlock (Conium maculatum), a plant containing high concentrations of coniine (2) and γ-coniceine (1) (Reynolds, 2005). In the popular literature, Agatha Christie continued this theme in a story plot involving a poison hemlock extract said to be high in coniine (2) as a means to commit murder in her book Murder in Retrospect (1984). Nicotine (7) in domestic tobacco (Nicotiana tabacum) as well as the pure alkaloid has been implicated in numerous cases of mild to severe acute toxicoses in humans (Gehlbach et al., 1974; Hagiya et al., 2010; Schep et al., 2009). Similarly, ingestion of lupines (Lupinus spp.) in the form of bitter lupine flour (principal toxicant: lupinine) and Laburnum spp. pods (principal toxicant: cytisine) have produced acute poisoning in humans (Pingault et al., 2009; Schep et al., 2009). Consumption of poisonous plants with high...
concentrations of piperidine alkaloids can produce acute intoxications in adult animals as well (Panter et al., 1999). Clinical signs of acute piperidine alkaloid toxicity in livestock include frequent urination, defecation, tachycardia, muscle weakness, muscle fasciculations, ataxia, collapse and ultimately death due to respiratory failure (Panter et al., 1988a,b).

The acute toxicoses produced by plant piperidine alkaloids have been attributed to their ability to desensitize nicotinic acetylcholine receptors (nAChRs) (Green et al., 2010). These receptor targets are ligand-gated cation channels, which mediate the actions of acetylcholine in excitatory neurotransmission within the peripheral and central nervous systems (Buccafusco, 2004). There are in excess of twenty subtypes of multi-subunit nAChRs, which are expressed in neurons and several non-neuronal cell types, including lymphocytes, skin cells and airway epithelia (Wessler and Kirkpatrick, 2008). Nicotine (7) and related alkaloids initially stimulate these receptors, but their persistence at their sites of action lead to receptor desensitization and inhibition of cation (Na+, Ca2+ and K+) conductances. Through their actions at nAChRs in the neuromuscular junction for example, nicotinoid alkaloids evoke transient skeletal muscle fasciculations that are rapidly followed by paralysis, progressing to respiratory failure. In adult animals, these muscle-type nAChRs consist of five associated subunits: two alpha, one beta, delta, and epsilon subunit that form a cation conductance channel. The two alpha-subunits are the main binding sites for acetylcholine and the adjacent delta and epsilon subunits play a complementary role in the receptor–ligand interaction (Arias, 2000). Fetal muscle-type nAChRs have a similar pentameric composition, except that they possess a gamma subunit in place of the epsilon subunit as the latter is expressed post-natally (Mishina et al., 1986).

As dramatic as acute toxicoses may be, sub-acute intoxication of pregnant livestock by piperidine alkaloids is a more common occurrence and has significant economic consequences to livestock producers (Keeler et al., 1993). Alkaloids ingested by the pregnant female can accumulate in the relatively more acidic blood of the conceptus (Neubert, 1988). Piperidine alkaloids, found in many poisonous plants including domestic tobacco, poison hemlock, wild tree tobacco (Nicotiana glauca), and lupine, can produce fetal deformities such as multiple congenital contractures (MCC) and cleft palates (CP) in the offspring of grazing livestock (Panter et al., 1990). MCC defects typically consist of arthrogryposis, scoliosis, torticollis, kyposis, and lordosis (Panter and Keeler, 1992, 1993). In utero exposure to piperidine alkaloids is not limited to livestock. Maternal tobacco use exposes the developing human fetus to nicotine (7), which has a myriad of adverse effects on fetal development and is considered to be a neuro-teratogen (for review see Bruin et al., 2010; Dwyer et al., 2009). These adverse development effects include congenital arthrogryposis, scoliosis, torticollis, kyposis, and lordosis (Panter and Keeler, 1992, 1993). All forms of arthrogryposis in humans and livestock manifest multiple joint contractures, attributable to decreased fetal movements during development (Fig. 2; Panter et al., 1990; Weinziegel et al., 2008). These multiple joint contractures were also produced in animal models by the administration of neuromuscular blocking agents such as d-tubocurarine, or specific piperidine alkaloids like anabasine (4) (Moessinger, 1983; Panter et al., 1999).

Cleft palates in cattle and goats can also result from the lack of fetal movements in the head and neck regions, resulting in the tongue preventing normal palate closure during early fetal or late embryo development. The resulting mechanical interference of the tongue between the palate shelves at the time of closure results in the formation of a cleft (Fig. 3; Panter and Keeler, 1992; Panter et al., 1998a). The period of gestation when a fetus is susceptible to these plant teratogens varies according to the

In utero exposure to piperidine alkaloids is not limited to livestock. Maternal tobacco use exposes the developing human fetus to nicotine (7), which has a myriad of adverse effects on fetal development and is considered to be a neuro-teratogen (for review see Bruin et al., 2010; Dwyer et al., 2009). These adverse development effects include congenital arthrogryposis, scoliosis, torticollis, kyposis, and lordosis (Panter and Keeler, 1992, 1993). All forms of arthrogryposis in humans and livestock manifest multiple joint contractures, attributable to decreased fetal movements during development (Fig. 2; Panter et al., 1990; Weinziegel et al., 2008). These multiple joint contractures were also produced in animal models by the administration of neuromuscular blocking agents such as d-tubocurarine, or specific piperidine alkaloids like anabasine (4) (Moessinger, 1983; Panter et al., 1999).

Cleft palates in cattle and goats can also result from the lack of fetal movements in the head and neck regions, resulting in the tongue preventing normal palate closure during early fetal or late embryo development. The resulting mechanical interference of the tongue between the palate shelves at the time of closure results in the formation of a cleft (Fig. 3; Panter and Keeler, 1992; Panter et al., 1998a). The period of gestation when a fetus is susceptible to these plant teratogens varies according to the

---

**Fig. 1.** Chemical structures of piperidine alkaloid teratogens. Alkaloids possessing a double bond between the nitrogen and the alpha carbon of the piperidine ring are on the left-hand side of the figure. The structure of the pyridine alkaloid nicotine, which does not cause MCC defects in livestock, is included for comparison.

**Fig. 2.** MCC defects in a yearling whose mother grazed lupine during 50–100 days of gestation (from Panter et al., 2009).
livestock species, the specific piperidine alkaloid, and the length of time exposed; but, in general, susceptible periods are 30–50 days for CP and 50–100 days for contracture defects of the forelimbs, spine and neck, and there must be continuous inhibition of fetal movement for defects to occur (Panter et al., 1992). Anabasine ([4]), a piperidine alkaloid that is associated with CP in goats (Panter et al., 1999), may similarly affect the developing human fetus in utero secondary to maternal tobacco use.

2. Structure–teratogenicity relationships of piperidine alkaloids

The initial work on structure–teratogenicity relationships of piperidine alkaloids from poisonous plants was performed by Keeler and Balls (Keeler and Balls, 1978). Using poison hemlock as a starting point, the researchers determined that conine ([2]) (2-propylpiperidine) and γ-coniceine ([1]) (2-n-propyl-L⁺-piperidine) could produce MCC defects in the offspring of pregnant cows dosed with plant that had high concentrations of either piperidine alkaloid. Both of these teratogens have a three-carbon side-chain alpha to the nitrogen of the piperidine ring with γ-coniceine ([1]) possessing a double bond between the nitrogen and the alpha carbon. The related alkaloids pipericidine, 2-methylpiperidine, 2-ethylpiperidine, 3-methylpiperidine, N-methylpiperidine and 2-piperidine–ethanol were not teratogenic in cattle. These results led the investigators to conclude that the position and length of the side-chain on the piperidine ring are critical for the formation of MCC defects, suggesting that piperidine alkaloids with a carbon side-chain of at least three carbons or larger attached to the carbon alpha to the piperidine nitrogen have teratogenic activity. The presence of a double bond adjacent to the nitrogen such as that found in γ-coniceine ([1]) is also thought to confer increased toxicity and teratogenicity. These observations were later confirmed using a mouse lethality bioassay with LD₅₀ values of 2.5 mg/kg for γ-coniceine ([1]), 11.4 mg/kg for coniine and 20.5 mg/kg for N-methyl coniine (Panter et al., 1998b).

Tobacco-induced deformities in pigs have also helped to expand the knowledge of piperidine alkaloid structure–activity/teratogenicity relationships. Reports of skeletal defects in the offspring of sows that grazed waste stalks of tobacco (Crowe, 1969; Crowe and Pike, 1973; Menges et al., 1970) led to tobacco feeding studies of pigs that reproduced the teratogenic syndrome observed in the field (Crowe and Swerczek, 1974). However, the pyridine alkaloid nicotine does not produce skeletal defects in livestock and was determined not to be the cause of the field outbreaks of skeletal defects in pigs (Crowe, 1978). This led to the hypothesis that other Nicotiana spp. alkaloids such as anabasine ([4]) are involved, as anabasine ([4]) was found at high concentration in the pulp of tobacco stalks and fit the structure–activity relationship proposed by Keeler and Balls (1978). Additional research with wild tree tobacco (N. glauca), which is naturally high in the piperidine alkaloid anabasine ([4]), found that it produced MCC defects in pigs and cows (Keeler et al., 1981a). In swine, anabasine ([4]) isolated from N. glauca produced MCC similar to those observed from N. tabacum (Keeler et al., 1984). These observations have been reproduced in cattle, sheep and goats (Panter et al., 1999; Keeler and Crowe, 1984; Keeler et al., 1981b).

Structure–teratogenicity relationships identified by Keeler and Balls (1978) and Keeler (1988) have led to a relative order for piperidine alkaloid teratogenicity (anabaseine ([3]) > anabasine ([4]) > coniine ([2]) > ammodendrine ([6])). Recently a pharmacodynamic comparison of piperidine alkaloids was made which led to the proposal of a teratogenic piperidine alkaloid activity profile (TAP; Green et al., 2010). The TAP is based on piperidine alkaloid structure, relative ruminant teratogenicity, relative rodent lethality, and activity at fetal muscle-type nAChRs constitutively expressed by TE-671 cells. Piperidine alkaloid induced reduction in fetal movement has been well documented by ultrasound experiments to cause both cleft palates and MCC deformities (Panter et al., 2000, 1990). The combination of results from ultrasound experiments and cell-based experiments, has led to the hypothesis that the mechanism behind MCC defects is the inhibition of fetal movements due to persistent desensitization of fetal muscle-type nAChRs by these alkaloids (Green et al., 2010). Normally, desensitization of nicotinic cholinergic receptors is a mechanism to protect against acetylcholine excitotoxicity over a short time scale (Giniatullin et al., 2005; Ochoa et al., 1989; Wang and Sun, 2005); however, if nAChR desensitization is prolonged by exposure to piperidine agonist alkaloids with considerably longer half-lives in the fetus, MCC defects or CP may occur. Results of recent experiments with TE-671 cells have identified a linear relationship between 50% effective concentration (EC₅₀, a measure of relative agonist potency) and percent of maximum electrical response of the cells (i.e. depolarization, a measure of agonist efficacy).
sugest that both enantiomeric forms of anabasine (4) are more potent teratogens than racemic anabasine (4) and have the potential to produce MCC in a developing fetus (Lee et al., 2006).

3. Specific piperidine alkaloids acting as teratogens

3.1. Ammodendrine (6) and N-acetylhystrine (5)

Ammodendrine (6) is a teratogenic piperidine alkaloid found in lupine species as a mixture of enantiomers; it is a partial agonist at nAChRs with an EC50 for the racemate that exceeds 500 μM in TE-671 cells (Table 1; Green et al., 2010; Daly, 2005; Lee et al., 2008a; Panter et al., 1994; Keeler and Panter, 1989). The 50% lethal dose (LD50) of ammodendrine (6) in a mouse-based bioassay ranges from 94 to 134 mg/kg depending on the form of the alkaloid (Table 1; Lee et al., 2005). N-Acetylhystrine (5) is also observed in L. formosus plant material by instrumental methods (Fitch et al., 1974); however, isolation for further characterization in cell based and animal models has not been completed because of the instability of N-acetylhystrine (5) in extraction and isolation procedures (Lee et al., 2005). N-acetylhystrine (5) is predicted to be a more potent teratogen than ammodendrine based on the relative teratogenicity model proposed by Keeler and Balls (1978). N-acetylhystrine (5) possesses a double bond between the nitrogen and the alpha carbon potentially resulting in increased teratogenic potency compared to ammodendrine (6) (Fig. 1).

3.2. Anabasine (4)

Anabasine (4) has been described as a “prototypical” piperidine nAChR agonist (Lesarri et al., 2010) and a nicotine-like natural product (Daly, 2005). Anabasine (4) is a potent agonist at fetal muscle-type nAChRs, with racemic anabasine (4) possessing an EC50 in the micromolar range to depolarize TE-671 cells (Table 1). This alkaloid is considered a minor tobacco alkaloid, is found at low concentrations in tobacco (N. tabacam), and is thought to act synergistically with other tobacco alkaloids in facilitating smoking behavior (Clemens et al., 2009). Anabasine (4) is in tobacco and tobacco products such as oral snuff, and cigarettes (Brunnemann et al., 2002; Wu et al., 2002; Liu et al., 2008; Huang and Hsieh, 2007; Djordjevic and Doran, 2009); as well as in biological samples from smokers (Miller et al., 2010a; Miller et al., 2010b; Jacob et al., 1999; Xu et al., 2004). The presence of anabasine (4) and anatabine in urine has been used as a biomarker of tobacco use (Jacob et al., 1999; Jacob et al., 2002). Anabasine (4) is the predominant piperidine alkaloid in tree tobacco (N. glauca), a plant that is often mistaken for wild spinach; its accidental consumption has been responsible for multiple fatalities in humans (Deboer et al., 2009; Castorena et al., 1987; Sims et al., 1999; Mellick et al., 1999; Mizrachi et al., 2000; Steenkamp et al., 2002; Furer et al., 2011). Anabasine (4) ingested in the form of dried ground tree tobacco produces MCC and cleft palate in calves, piglets, lambs and goat kids (Panter et al., 1990; Panter and Keeler, 1992; Keeler et al., 1984; Keeler and Crowe, 1984; Keeler et al., 1981b; Keeler and Crowe, 1983).

3.3. Anabaseine (3)

Anabaseine (3) is a potent agonist at fetal and adult muscle-type and neuronal nAChRs; it has an EC50 in the nanomolar range in TE-671 cells (Table 1; Green et al., 2010; Lee et al., 2008; Hunter et al., 1994; Kem, 1997). The alkaloid has been identified in tobacco (Duffield et al., 1965) and appears to be a degradation product of (±)-anabasine (4) in the plant (Kisaki and Tamaki, 1966). Like anabasine (4), anabaseine (3) has been classified as a minor tobacco
alkaloid (Dowskin et al., 1995). Anabasine (3) has been found in nemertean worms (Kem, 1971), Aphaenogaster ants (Wheeler et al., 1981), in the poison gland of Cape harvester ants (Messor capensis) (Brand and Mpu, 1993), and in the scent gland secretions of the European Cladonychiid harvestman (Holoscotolemon lessiniense) (Raspotnig et al., 2011). Anabasine (3) shares a double ring structure with anabasine (4), and has a double bond between positions 1 and 2 of the piperidine ring (Fig. 1). The double bond between the nitrogen and the alpha carbon increases its toxicity and teratogenicity (Panter et al., 1999; Lee et al., 2006). Due to its relatively high potency at neuronal nAChRs, anabasine (3) has been used as a structural base for the synthesis of selective alpha7 neuronal nAChR agonists, which are designed to improve cognitive function in patients with Alzheimer’s disease (Kem et al., 1997; Arias et al., 2009).

4. Summary and implications for human health

Piperidine alkaloids from poisonous plants have provided insights into the mechanisms underlying MCC defects and CP in livestock and humans. They accumulate in fetal blood and act at fetal nAChRs, which may be more susceptible to them than adult receptors. Their teratogenic actions are hypothesized to involve persistent nAChR desensitization; leading to an inhibition of fetal movements. Through this proposed mechanism, livestock teratogens such as anabasine (4) and anabasine (3) could also carry teratogenic risks in humans due to potential exposure from tobacco consumption and/or tobacco replacement therapy (Slotkin, 2008). Tobacco use by pregnant women is associated with many types of birth defects including cleft palates and lips (Shaw et al., 2009; Hackshaw et al., 2011). A recent meta-analysis by Hackshaw et al. (2011) calculated an odds ratio of 1.28 indicating a 28% increased incidence of cleft lip or palate in children from mothers that used tobacco during pregnancy. Moreover, an epidemiological survey of eleven states in the US estimated the prevalence of these defects at 6.39–10.48 per 10,000 live births (NIDCR, 2011). This represents approximately 6000–7000 live births with palate or lip defects annually in the United States (NIDCR, 2011). A greater understanding of the mechanism(s) behind oral cleft formation in humans will have significant impact in reducing their incidence, decreasing overall medical costs and the emotional impacts to families and individuals. For example, nicotine replacement therapy is currently being investigated for use by pregnant smokers,
although, it is not without controversy (Forest, 2010; Coleman et al., 2011). For women who are unable to quit using tobacco during pregnancy, nicotine (7) replacement therapy may provide a means to prevent the exposure of teratogenic piperidine alkaloids to the developing fetus and thereby reduce the risk of MCC defects and oral clefts in their children. The premise of this recommendation is based on in vitro results from cell-based studies of human fetal muscle-type nAChR expressed by TE-671 cells, in vivo results from the exposure of pregnant livestock to piperidine alkaloid teratogens, and human epidemiological data which have documented an association between tobacco use by pregnant women and oral clefts in their children. Continued and expanded research on the molecular mechanisms behind the teratogenic effects of piperidine alkaloids is needed, and will provide benefits for both humans and livestock through decreased associated medical costs, improved livestock production systems, and associated economic gains to agricultural producers.

Conflict of Interest

There are no conflicts of interest associated with this work.

Acknowledgements

The authors thank Terrie Wierenga for expert assistance with the figures and Isabelle McCollum and Amber Whittaker for assistance with the manuscript. This work was supported by USDA/ARS. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

References


