Efficacy of ERL-4221 as an ovotoxin for feral pigs (*Sus scrofa*)

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Abstract

Context. The expansion of feral pig populations across the United States has increased the occurrence of damage and damage complaints. New techniques are needed to more effectively manage feral pig damage, including the development of fertility control agents.

Aims. We aimed to assess the ovotoxic properties of ERL-4221 as a candidate fertility control agent for feral pigs.

Methods. We conducted two palatability trials to determine ERL-4221 acceptance and one experimental trial with ERL-4221 at the captive wildlife facility of Texas A&M University-Kingsville during 2008. Our experimental trial had three treatments, a control containing no ERL-4221, baits containing 16.0 mg ERL-4221 kg^{-1} bodyweight for 10 days, and baits containing 16.0 mg ERL-4221 kg^{-1} bodyweight for 20 days.

Key results. Final body mass, total ovary mass, number of follicles and number of corpora lutea did not differ between treatments.

Conclusions. We did not find it efficacious to orally deliver ERL-4221 to feral pigs to reduce fertility. Oral delivery is the most practical, cost-effective means of delivering fertility control agents to feral pigs and development of additional fertility control strategies are needed.

Implications. Unless ovotoxic effects of ERL-4221 can be identified in feral pigs, along with a successful means of administration, other fertility control strategies may need to be explored, such as oocyte-secreted proteins that regulate follicular development.

Additional keywords: ERL-4221, fertility control, oocyte, ovary, toxicity.

Introduction

The recent expansion of feral pig (*Sus scrofa*) populations across the United States has increased the occurrence of damage and damage complaints by producers, natural resource managers and biologists (Adams *et al*. 2005). Existing techniques to control feral pig damage in the United States include fencing, diversion, trapping and shooting (Campbell and Long 2009). New techniques are needed to more effectively manage feral pig damage, including the development of toxicants (Cowled *et al*. 2008) and fertility control agents (Campbell *et al*. 2010).

4-Vinyl-1-cyclohexene diepoxide (VCD, CAS# 106–87–6) is an industrial chemical used as a synthetic intermediate and reactive diluent for diepoxides and epoxy resins (Mauldin and Miller 2007). The compound is ovotoxic and has been used extensively to study the process of menopause in humans using mice and rats as biological models (Hoyer *et al*. 2001).

The compound destroys the oocytes contained within preantral follicles in the ovary, a process thought to be caused by a reduced ability to convert the epoxide to an inactive tetrol in the oocyte (Hoyer *et al*. 2001). In 2005, the industrial use of VCD was discontinued, both reducing supply and significantly increasing the cost of the compound. Another diepoxide, cycloaliphatic epoxide resin (ERL-4221, CAS# 2386–87–0), replaced VCD in industrial usage primarily because of lower toxicity, carcinogenicity, volatility and cost (Mauldin and Miller 2007). However, there are no reports on the efficacy of ERL-4221 as an ovotoxin and therefore it is unknown whether it is a possible candidate as a fertility control agent.

Our objectives were to assess the ovotoxic properties of ERL-4221 as a candidate fertility control agent for feral pigs and to determine the feasibility of orally delivering ERL-4221 to feral pigs. Because ERL-4221 is structurally similar to VCD (Mauldin and Miller 2007), we predicted that ERL-4221, when delivered on

a molar concentration basis comparable to VCD levels, might reduce folliculogenesis and ovulation of feral pigs.

Materials and methods

Our study was conducted at the captive wildlife facility of Texas A&M University-Kingsville, located 1.5 km north of Kingsville, Texas. In April 2008, we placed wild caught female feral pigs in individual covered pens (3.0×3.7 m), where they were maintained throughout the trials. Prior to our trials we collected a blood sample from each animal, separated serum by centrifugation, used the pseudorabies virus (PRV) antibody test kit (Viral Antigens Inc., Memphis, TN) to test for antibodies against PRV, and the buffered *Brucella* antigen card test (United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Washington, DC) to test for antibodies against *Brucella*. We did not use antibody-positive pigs in our trials. Water and commercial feed (USDA Pig, Lyssy and Eckels, Poth, TX) were available to animals throughout the study, except during 4 h fasting periods. All animal handling, care and use procedures were approved by the Institutional Animal Care and Use Committee at Texas A&M University-Kingsville (permit number 2007–07–20).

We conducted two palatability trials to determine ERL-4221 acceptance and one experimental trial. Throughout these we used strawberry flavoured baits (175–205 g) with varying concentrations of ERL-4221. For all trials, each pig received an amount of bait that, if it was all consumed, gave it the specified dose for its actual bodyweight. We added strawberry flavouring (Strawberry Aroma, QualiTech, Chaska, MN) to increase palatability (Campbell and Long 2008). Experimental baits were made at the National Wildlife Research Center and consisted of water, calcium carbonate, surfactant, corn oil, sugar, flavouring, konjac and ERL-4221, where applicable.

For palatability trial 1, which encompassed 13 days, we used three feral pigs. We fasted pigs daily from 0700 to 1100 hours, then introduced baits of known weight at ~1105 hours and monitored baits for consumption for ≤ 1 h. Baits on days 1–4, 6, 8, 10 and 12 contained no ERL-4221. Baits on days 5, 7, 9, 11 and 13 contained 7.8, 15.6, 31.25, 62.5 and 125 mg ERL-4221 kg^{-1} bodyweight, respectively.

To further understand palatability relationships of baits containing ERL-4221, we conducted a second 7-day palatability trial with the same three feral pig individuals. We again fasted animals from 0700 to 1100 hours, then introduced baits of known weight at ~1105 hours and monitored baits for consumption for ≤ 1 h. Baits on days 1, 2, 4 and 6 contained no ERL-4221. Baits on days 3, 5 and 7 contained 15.6, 20.0 and 25.0 mg ERL-4221 kg^{-1} bodyweight, respectively.

We designed our experimental trial using acceptable ERL-4221 levels determined from the palatability trials. Our experimental trial used 17 feral pigs randomly assigned to one of three treatments. Our control treatment ($n = 5$) consisted of a daily ration of a bait containing no ERL-4221 for 20 days. Our short-duration treatment ($n = 6$) consisted of a daily ration of a bait containing no ERL-4221 for 10 days followed by a daily ration of a bait containing 16.0 mg ERL-4221 kg^{-1} bodyweight for 10 days. Our long-duration treatment ($n = 6$) consisted of a

daily ration of a bait containing 16.0 mg ERL-4221 kg^{-1} bodyweight for 20 days. To accomplish this we fasted animals from 0700 to 1100 hours daily, then introduced baits of known weight at ~1105 hours and monitored baits for consumption for ≤ 1 h. Following the treatment period (20 days), we maintained feral pigs in all treatments groups for an additional 30 days in a post-treatment recovery period. At the end of this period we euthanised the animals with a penetrating captive bolt following approved guidelines (AVMA 2007) and weighed the carcasses. We collected ovaries and determined mass. We then stored ovaries in 10% formalin. We summed these data from right and left ovaries and report means ($\pm$s.e.) with 95% confidence intervals by treatment.

We quantified the number of primary, secondary, tertiary and pre-ovulatory follicles and the number of corpora lutea on each ovary. Because we did not detect gross morphological differences between right and left ovaries, we analysed only the right ovary for follicular development. We divided formalin-saturated ovarian tissue into 1 mg slices, which we dehydrated through a graded series of ethanol solutions (40, 60, 80, 95 and 100%) for 1 h each at 4°C. We transferred tissue from 100% ethanol solution to xylene for 1 h. We placed the tissue from xylene into a paraffin bath (TissuePrep, Fisher Scientific, Pittsburgh, PA) at 60°C for 2 h and then we embedded tissue in a cassette mould. We sectioned tissue to 6 μm and placed tissue on slides (ProbeOn Plus slides, Fisher Scientific) with two serial sections per slide. Following the sectioning process, we deparaffinised and rehydrated tissue through a graded series of ethanol solutions (100, 100, 95, 95 and 70%) for 2 min each. We stained tissue sections with hematoxylin dipped in graded series of ethanol (79, 95 and 100%) for 15 s each and then xylene for 1 min. We dried and mounted sections with Permount (Fisher Scientific) for permanent fixation.

We randomly selected three areas of 13.3×10^4 mm^2 slide^{-1} (six slides per tissue block) to quantify follicles and corpora lutea. We located follicles in the cortex of ovarian tissue and categorised these by stage of development following Oxender *et al.* (1979) and Griffin *et al.* (2006): primary follicles, secondary follicles, tertiary follicles and pre-ovulatory follicles. We report mean ($\pm$s.e.) number of follicles and corpora lutea with 95% confidence intervals by treatment.

Results

During our palatability and experimental trials, feral pigs either consumed all or none of the bait and that no partial consumption was observed. However, animals encountering baits containing >16.0 mg ERL-4221 kg^{-1} bodyweight often displaying excessive salivation and rigorous head shaking, followed by body rolls on top of the baits.

During palatability trial 1, all feral pigs consumed baits containing 7.8 mg ERL-4221 kg^{-1} bodyweight, two pigs consumed baits containing 15.6 mg ERL-4221 kg^{-1} bodyweight, and one consumed bait containing 31.25 mg ERL-4221 kg^{-1} bodyweight. During this trial no animals consumed baits containing 62.5 mg ERL-4221 kg^{-1} bodyweight. During palatability trial 2, all feral pigs consumed baits containing 15.6 mg ERL-4221 kg^{-1} bodyweight and no animals consumed baits containing 20.0 or 25.0 mg ERL-4221 kg^{-1} bodyweight. We

concluded that a palatability threshold exists between 15.6 and 20.0 mg ERL-4221 kg^{-1} bodyweight, above which feral pigs generally will not consume baits.

During our experimental trial, final body mass of individuals in control (42.9 kg, 95% CI 29.5–56.3), 10-day (46.7 kg, 95% CI 39.6–53.8) and 20-day treatments (42.8 kg, 95% CI 35.0–50.6) were not statistically different (Fig. 1). Similarly, total ovary mass in control (3.68 g, 95% CI 1.74–5.62), 10-day (3.82 g, 95% CI 2.60–5.03) and 20-day treatments (2.92 g, 95% CI 2.29–3.54) did not differ (Fig. 2). The number of follicles detected in control, 10-day and 20-day treatments did not differ (Fig. 3). Lastly, the number of corpora lutea detected in control (5.3, 95% CI 2.8–7.8), 10-day (4.4, 95% CI 2.1–6.7) and 20-day treatments (3.0, 95% CI 0.4–5.6) were not different (Fig. 4).

Discussion

In mammals, females are born with a finite number of ovarian primordial follicles containing primary oocytes, which cannot be regenerated when lost (Hoyer *et al*. 2001). Over the female's lifetime, >99% of these primary oocytes will undergo natural

atresia (Mayer *et al*. 2004). Due to the inability of the mammalian ovary to replace atretic follicles, the administration of an ovotoxin provides the opportunity to permanently sterilise a female by destroying oocyte-containing primordial and primary follicles (Hoyer *et al*. 2001).

For example, in mice and rats, VCD is administered experimentally by daily intraperitoneal injections at concentrations ranging from 40 to 320 mg kg^{-1} bodyweight for about two weeks (Devine *et al*. 2004; Mayer *et al*. 2004; Thompson *et al*. 2005). Complete or near-complete eradication of ovarian oocytes is achieved at higher doses (Devine *et al*. 2004). Additionally, female dogs dosed subcutaneously daily for six days with VCD at concentrations ranging from 80 to 240 mg kg^{-1} bodyweight showed a decrease in the primordial follicle pool 30 days after dosing initiation (Miers *et al*. 2005). These studies suggest promise for VCD and other diepoxides, such as ERL-4221, as candidate fertility control agents (Mauldin and Miller 2007).

In real-world sterilisation of wild animals, oral administration is a more vaible delivery method than
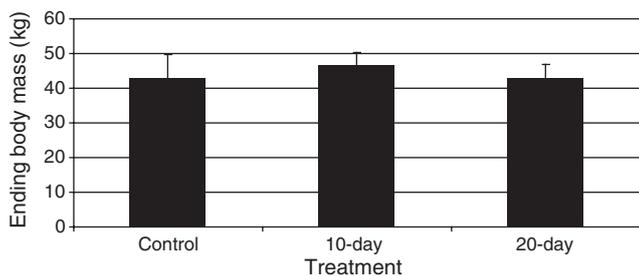

Fig. 1. Mean (±s.e.) final body mass (kg) of female feral pigs in control (no ERL-4221), 10-day and 20-day treatment groups at the captive wildlife facility of Texas A&M University-Kingsville during 2008. Pigs in the 10-day treatment were fed baits with 16.0 mg ERL-4221 per kg bodyweight for 10 days. Pigs in the 20-day treatment were fed baits with 16.0 mg ERL-4221 per kg bodyweight for 20 days.
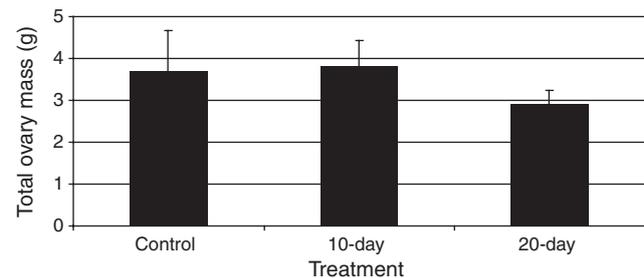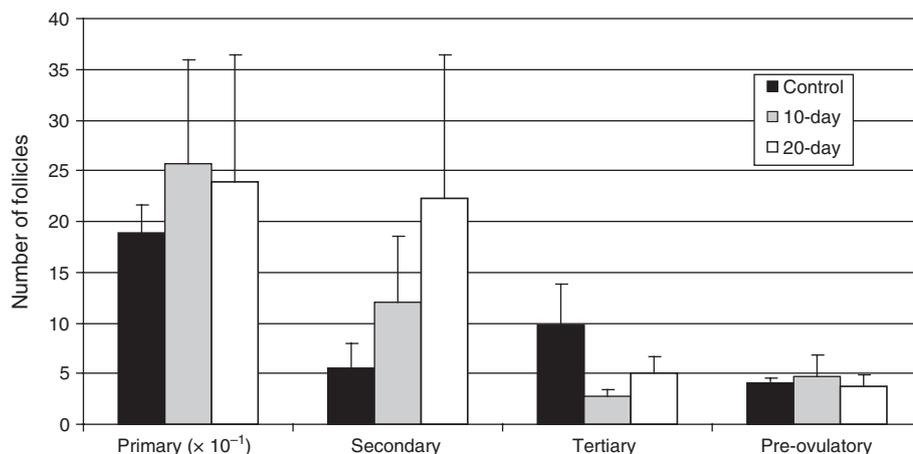

Fig. 2. Mean (±s.e.) total ovary mass (g) of feral pigs in control (no ERL-4221), 10-day and 20-day treatment groups at the captive wildlife facility of Texas A&M University-Kingsville during 2008. Pigs in the 10-day treatment were fed baits with 16.0 mg ERL-4221 per kg bodyweight for 10 days. Pigs in the 20-day treatment were fed baits with 16.0 mg ERL-4221 per kg bodyweight for 20 days.
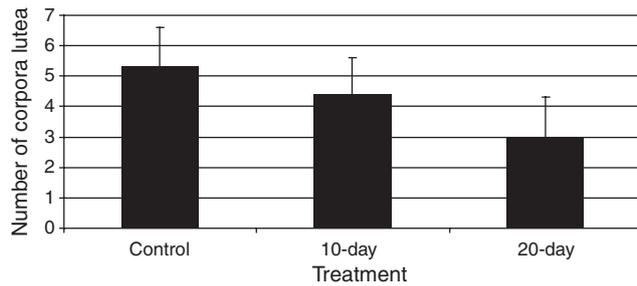

Fig. 3. Mean (±s.e.) number of primary (×10^1), secondary, tertiary and pre-ovulatory follicles of feral pigs in control (no ERL-4221), 10-day and 20-day treatment groups at the captive wildlife facility of Texas A&M University-Kingsville during 2008. Pigs in the 10-day treatment were fed baits with 16.0 mg ERL-4221 per kg bodyweight for 10 days. Pigs in the 20-day treatment were fed baits with 16.0 mg ERL-4221 per kg bodyweight for 20 days.

Fig. 4. Mean ($\pm$s.e.) number of corpora lutea of feral pigs in control (no ERL-4221), 10-day and 20-day treatment groups at the captive wildlife facility of Texas A&M University-Kingsville during 2008. Pigs in the 10-day treatment were fed baits with 16.0 mg ERL-4221 per kg bodyweight for 10 days. Pigs in the 20-day treatment were fed baits with 16.0 mg ERL-4221 per kg bodyweight for 20 days.

catching and injecting wild animals. In addition, for this trial, catching wild pigs every day for up to 15 days would have been very difficult. Feral pigs accepted baits containing $\leq$16.0 mg ERL-4221 kg^{-1} bodyweight, a concentration >2.5 times less than that used in other studies (Devine *et al*. 2004). Therefore, we included a longer duration experimental treatment of 20 days. We surmise that our low bait palatability was due to ERL-4221 negatively stimulating the trigeminal nerve of feral pigs. For example, ERL-4221 causes a burning sensation in the oral cavity (R. E. Mauldin, National Wildlife Research Center, pers. comm.).

Twenty days of oral administration of ERL-4221 at 16.0 mg kg^{-1} bodyweight did not induce any signs of ovotoxicity in feral pigs. Our negative results may be because ERL-4221 is not ovotoxic to pigs, we were not able to deliver sufficient concentrations of ERL-4221 to pigs due to low palatability, we did not expose pigs to ERL-4221 for a long enough duration, or the digestive or metabolic processes in pigs reduces the ovotoxicity of ERL-4221. For example, exposure of ERL-4221 to stomach pH may have hydrolysed the epoxide groups. Unless these limitations can be addressed, ERL-4221 appears to be an ineffective chemical for fertility control in wild pigs. Regardless, oral delivery is the most practical, cost-effective means of delivering fertility control agents to wild animals (Fagerstone *et al*. 2006). In this study, ERL-4221 baits did not produce sterility in feral pigs. Data suggest that fertility control methods targeting males only are likely to be inefficient if female promiscuity is high (Delgado-Acevedo *et al*. 2010); methods targeting females or both sexes jointly may be more effective. Existing fertility control strategies are available for several avian species (e.g. DiazaCon™ and Nicarbazin) and white-tailed deer (GonaCon™).

Conclusions

In these trials, it was not efficacious to orally deliver ERL-4221 to feral pigs to reduce fertility. Unless the ovotoxic effects of ERL-4221 can be identified in wild pigs, along with a successful means of administration, other fertility control strategies may need to be explored. For instance, a recently developed strategy involves oocyte-secreted proteins that regulate follicular development (Mauldin and Miller 2007). New tools like these may lead to effective control of damaging feral animal populations, such as wild pigs.

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