Transmucosal administration of pentobarbital and phenytoin solution induces euthanasia in bearded dragons (Pogona vitticeps)

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OBJECTIVE
To assess the efficacy of transmucosal euthanasia solution to induce euthanasia.

ANIMALS
6 bearded dragons (Pogona vitticeps).

METHODS
An initial dose of euthanasia solution containing pentobarbital and phenytoin sodium was administered transmucosally in conscious lizards (100 mg/kg pentobarbital dose), followed by a second dose 20 minutes later (400 mg/kg pentobarbital dose). The presence of movement, leakage of euthanasia solution, behaviors consistent with oral irritation, respiratory rate, heart rate, palpebral and corneal reflex, and response to noxious stimuli were recorded until death, confirmed by the absence of Doppler cardiac flow and cardiac electrical activity. The time to loss of all parameters was calculated. Postmortem evaluation allowed for histopathologic evaluation of the oral cavity and gastrointestinal tract to detect potential mucosal damage from the alkaline euthanasia solution.

RESULTS
The median time to death was 300 minutes (range, 300 to 360 minutes), median time to respiratory arrest was 30 minutes (range, 30 to 50 minutes), and median time to loss of deep pain response was 30 minutes (range, 20 to 50 minutes). Signs consistent with oral irritation occurred in 4 of 6 (66.7%) lizards, including 2 lizards that exhibited whole-body spasms after euthanasia solution administration. Histopathologic changes indicating peracute mucosal ulceration, suspected to be from caustic causes, were identified in 1 (1/6 [16.7%]) lizard.

CLINICAL RELEVANCE
Transmucosal euthanasia solution administration resulted in clinical euthanasia within 6 hours. This method should be utilized only after premedication with analgesic and/or anesthetic medications due to the potential for acute mucosal ulceration and behaviors that may be distressing in client-owned animals.

Keywords: bearded dragon, pogona vitticeps, euthanasia, pentobarbital, transmucosal
death. Thus, a secondary physical method, such as decapitation, pithing, freezing, or IV injection of potassium chloride, is recommended to confirm death. In the case of companion reptiles, many of these techniques may be regarded as unpleasant to clients; thus, exploration of further protocols that do not require a secondary method and still provide effective and discomfort-free euthanasia is needed.

Pentobarbital is a short-acting barbiturate that functions by binding to the GABA receptor-ionophore complex. This results in prolonged inhibitory effects on the CNS. Overdose leads to rapid sedation, followed by respiratory and cardiovascular depression, then progresses to coma and death. It is almost odorless and has a slightly bitter taste to humans. Pentobarbital is highly soluble in water and alcohol; in 10% solution in water, it is highly alkaline, with a pH of 9.6 to 11. Phenytoin is an anticonvulsant drug commonly mixed with pentobarbital in euthanasia solutions. The mechanism of action is not well understood, but phenytoin ultimately leads to CNS depression. Tasteless and nearly odorless, it is acidic, with a pH of 2.7, and is soluble in water at 22 °C (71.6 °F).

This prospective study investigated the effects of transmucosal administration of euthanasia solution in bearded dragons and builds on prior work performed in aquatic chelonians and wild birds. It was hypothesized that transmucosal pentobarbital would induce euthanasia without the need for a secondary method within 24 hours. It was also hypothesized that oral and upper gastrointestinal mucosal damage would be detected histologically on postmortem evaluation due to irritation from highly alkaline euthanasia solution.

Methods

Animals
Six bearded dragons (4 males and 2 females; weight range, 0.31 to 0.39 kg [0.67 to 0.85 lbs]) maintained as a teaching and research colony were enrolled. Bearded dragons were 5 years old at the time of this study, and procedures were performed in accordance with an approved IACUC (#22209).

Two hours prior to euthanasia administration, all external heat sources were removed, and the ambient room temperature, maintained between 23.3 to 24.4 °C (74.0 to 76.0 °F), was recorded using 2 separate thermometers. During the entirety of the study, the lizards were individually housed in 91.5 X 71.1 X 45.7-cm enclosures, all located in the same temperature-controlled room. The lizards were fasted overnight with ready access to water.

Administration of euthanasia solution
Each lizard was manually restrained in sternal recumbency, and the oral cavity was digitally opened to allow monitoring of leakage of the solution from the oral cavity.

An initial dose of pentobarbital (100 mg/kg; range of volume delivered, 0.08 to 0.10 mL) was administered to induce a generalized anesthetized state, and after 20 minutes a second dose of pentobarbital (400 mg/kg; range of volume delivered, 0.31 to 0.40 mL) was delivered in the same manner. The phenytoin doses were 13 mg/kg for the initial dose and 51 mg/kg for the second dose. These doses were selected based on previous reptile euthanasia studies and to result in practical administration volumes for the initial dose, being cognizant that this dose necessitated administration to conscious lizards. After handling, the lizards were left in their individual enclosures on clean newspaper substrate to allow monitoring of leakage of the solution from the oral cavity.

Observations
After the delivery of the initial dose of euthanasia solution, serial observations were recorded until confirmation of death. Observations recorded included the presence or absence of movement without stimulus, movement with stimulus, any visible leakage of pentobarbital from the oral cavity, clawing at the mouth or yawning, respiratory rate (RR), heart rate (HR), the presence or absence of palpebral reflex and corneal reflex, and response to noxious stimuli (superficial and deep pain). Observations were made starting 10 minutes after initial euthanasia solution administration and every 10 minutes thereafter (first 60 minutes), then every 60 minutes (next 5 hours), then every 4 hours (until 24 hours).

Movement without stimulus was defined as present if the lizard moved independently of any handling or physical stimulation. Movement with stimulus was defined as present if any visible movements were made by the bearded dragon during handling, positioning of the Doppler probe for HR recording, or stimulation for the evaluation of palpebral reflex, corneal reflex, or pain sensation. Leakage from the oral cavity was defined as solution (pink liquid consistent with the color of the euthanasia solution) visibly present external to the oral cavity or present on the newspaper substrate after administration. Clawing at the mouth was defined as movement of the thoracic limbs to rub the oral cavity or face. Yawning was defined as opening the mouth. RR was measured via direct visualization of respiratory movements before handling and recorded over 30 seconds. The HR was measured by placing a Doppler probe in the axillary region and recording over 30 seconds; if no HR was detected, the monitoring was continued for 120 seconds. Positive response for palpebral reflex was defined as the movement of the superior or inferior palpebrae in response to touching a cotton-tipped applicator at the medial and lateral canthi of the eye. If the palpebral reflex was absent, the corneal reflex was tested on the same eye by using a cotton-tipped applicator moistened with 0.9% saline to touch the corneal surface and evaluate for the presence of movement of the superior or inferior palpebrae. For both palpebral and corneal reflexes, the eye tested (left eye or right eye) was randomized for
each observation at each time point. The presence of a superficial pain response was defined as positive if the limb was withdrawn in response to a noxious stimulus (pinching of interdigital skin using a hemostat); the absence of superficial pain was defined as no reaction occurring as the hemostat pinch was held for 5 seconds. If the superficial pain response was absent, deep pain response was assessed on the same limb by using hemostats to pinch over the metacarpal or metatarsal bones. A positive deep pain response was defined as a limb withdrawal reaction within 5 seconds and defined as absent if no reaction occurred within this time.

Randomization of which limb and eye were sampled at each time point for each lizard was assigned a priori using the RAND function from commercial spreadsheet software (Excel 2024, version 16.81; Microsoft Corp). For limb assignment, the software was asked to randomly assign a number between 1 and 4 for each sampling time and each lizard, with each number representing 1 of 4 limbs available. Similarly, for eye assignment, the software was asked to randomly assign a number between 1 and 2 for each sampling time and each lizard, with each number representing 1 eye.

Confirmation of death
Once there was no detectable RR, HR, voluntary movement, reflexes, or response to noxious stimuli, ECG leads were placed in standard lead II positioning using patient side electrocardiography (VetCorder Pro, Sentier HC LLC). Death was confirmed when no electrical conduction was present after at least 5 minutes of recording; at that time, no further observations were recorded, and the body was left in the individual housing until necropsy was performed.

Histopathology
Twenty-four hours after administration of euthanasia solution and 19 to 24 hours after death was confirmed, the celom of each lizard was incised on the ventral midline for gross celomic evaluation. Following this, the entire carcass was immersed in 10% neutral-buffered formalin for fixation. Cross-sections through the tongue, oropharynx, and cranial esophagus were collected and routinely processed for histology. Tissue sections were embedded in paraffin, sectioned 5-µM thick, mounted on glass slides, and stained with H&E. Slide-mounted and H&E-stained tissue sections were evaluated by a board-certified veterinary anatomic pathologist (D.M.I.).

Statistical analyses
Time to loss of all observations (movement with or without stimuli, RR, HR, palpebral reflex, corneal reflex, superficial pain, deep pain) was recorded, with time 0 represented as administration of the initial pentobarbital dose. The range and median were calculated for time-to-loss data using the spreadsheet software. Nonparametric testing was performed given the small sample size.

Results
Observations
Leakage of euthanasia solution was observed in 4/6 (66.67%) lizards. Leakage was observed in 1 lizard 30 minutes after initial euthanasia solution administration (10 minutes after the second administration). At 60 minutes, 3 lizards were noted to have leakage. No clawing at the mouth or yawning were observed in any of the 6 lizards. However, at 0 minutes, 4 lizards (66.67%) demonstrated lip smacking, heavy breathing, and repeated swallowing after initial euthanasia solution administration. One of these lizards additionally demonstrated sudden, violent whole-body spasms concurrently. At 20 minutes, following administration of the larger dose of euthanasia solution, 2 lizards demonstrated this same behavior. One of these lizards was the same individual that exhibited this behavior at 0 minutes.

No lizard regained movement (with or without stimulus), respirations, or palpebral or corneal reflexes after initially losing these parameters. One lizard lost superficial pain at 10 minutes, regained it at 20 minutes, and finally lost it again at 30 minutes (bearded dragon 2). Another lizard had no deep pain response at 20 or 30 minutes but responded at 40 minutes. This lizard finally lost response to deep pain at 50 minutes (bearded dragon 3). The last time the responses were noted were recorded as these lizards’ time to loss for that variable (Table 1).

<p>| Table 1—Individual and median time to loss of observations and time to death recorded from 6 bearded dragons (Pogona vitticeps) administered transmucosal euthanasia solution containing pentobarbital and phenytoin. |
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<th>Individual bearded dragons</th>
<th>Time to loss of observations (min)</th>
<th>Movement without stimulus</th>
<th>Movement with stimulus</th>
<th>RR</th>
<th>HR</th>
<th>Palpebral reflex</th>
<th>Corneal reflex</th>
<th>Superficial pain</th>
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Time of death was defined as the time when a lack of cardiac Doppler flow and ECG activity was recorded. HR = Heart rate. RR = Respiratory rate.
A lizard lost HR at 2 hours, then exhibited pulseless electrical activity until 5 hours (bearded dragon 3). Another lizard had no detectable heartbeat at the 20-minute, 50-minute, and 60-minute time points, exhibited pulseless electrical activity during these recording periods, then regained a detectable heartbeat at 360 minutes (6 hours, bearded dragon 1). Thus, the time of death, determined by lack of cardiac Doppler flow and ECG activity simultaneously, ranged from 300 minutes (5 hours) to 360 minutes (6 hours), with a median of 300 minutes (5 hours).

Postmortem evaluations

Upon necropsy, none of the hearts exhibited contractility at rest or when gently prodded, and the celom of the lizards was dominated by gas distention of the bowel. Grossly, the mucosal surfaces of the tongue and oropharynx had no evidence of erythema or ulceration.

Histopathology of the tongue identified abnormalities in 3 lizards (3/6 [50%]): regional mucosal bacterial overgrowth (1/6 [16.67%]), regional asymmetric myodegeneration and atrophy (1/6 [16.67%]), and mild chronic glossitis (1/6 [16.67%]). Two lizards had oropharyngeal and esophageal abnormalities including mild, multifocal to regionally extensive, peracute erosion (1/6 [16.67%]) and mild chronic erosion (1/6 [16.67%]). Other gastrointestinal findings included minimal chronic lymphocytic gastritis (1/6 [16.67%]), mild lymphocytic to histiocytic enteritis (1/6 [16.67%]), and mild heterophilic to histiocytic colitis (2/6 [33.33%]). Of these findings, the only relevant histopathological change that could represent potential acute caustic irritation from the euthanasia solution was the peracute oropharyngeal/esophageal mucosal erosion. This finding was identified in the lizard that demonstrated whole-body spasms at 0 and 20 minutes.

Discussion

Euthanasia is defined by AVMA as a process causing minimal distress with rapid loss of consciousness leading to death. The timeframe in which “rapid” loss of consciousness is achieved in reptiles is not well defined. In this study, the median time to loss of deep pain response (and thus a generalized anesthetized state) was 30 minutes. One lizard that lost deep pain response at 30 minutes regained it at 40 minutes, and this either represents a true return to consciousness or an error in data collection (eg, inadequate initial noxious stimulation) at the 30-minute time point. This lizard did not regain deep pain response after losing it again at 50 minutes. Time to loss of deep pain response in this cohort was prolonged compared to a transmucosal pentobarbital study in pond slider turtles (Trachemys scripta) and an intracelomic/intracardiac pentobarbital study in leopard geckos (Eublepharis macularius). The chelonians lost deep pain response at 15 minutes postadministration, and the leopard geckos lost deep pain response within 27 minutes. While differences in drug metabolism exist between species, this contrast is likely attributable to methodology: the pond slider turtles were maintained at their preferred optimal temperature zone, whereas the bearded dragons in this study were kept at a cooler ambient temperature. Additionally, the leopard geckos were administered higher doses of pentobarbital (400 or 800 mg/kg intracelomic, 800 mg/kg intracardiac) compared to those used in this study. Maintenance of study bearded dragons at their preferred temperature range (29 to 31°C [84 to 88°F]) may have permitted faster metabolism of euthanasia solution and may have potentially resulted in more rapid loss of deep pain response. In our study, we chose to maintain the lizards at a lower temperature to closer approximate the ambient room temperature likely experienced in clinical practice where lizards are euthanized. In addition, higher doses of pentobarbital were not pursued to achieve realistic delivery volumes to awake lizards.

Behavior that may be interpreted as distress and/or pain occurred in 4 lizards prior to unconsciousness (4/6 [66.67%]). These behaviors included lip smacking, heavy breathing, repeated swallowing, and whole-body spasms. The lip smacking, heavy breathing, and repeated swallowing could be attributed to the bitter taste of pentobarbital. This euthanasia solution has been demonstrated to be distasteful to laboratory mice based on poor voluntary ingestion when mixed into cookie dough. These behaviors were not observed in a prior study in pond sliders administered euthanasia solution via oral gavage. However, wild avian species administered transmucosal euthanasia solution exhibited head shaking and ticks, vocalization, tremors, and wing flapping. Contact irritation from the alkaline solution may have occurred, but there was no histologic evidence of acute mucosal erosion in 3 of the 4 lizards demonstrating lip smacking, heavy breathing, and repeated swallowing behaviors. The prior reports in pond turtles and wild birds did not report on histopathologic changes. Prior investigations into the effect of alkaline solutions in laboratory rats yielded conflicting results: acute gastritis and hemorrhage occurred in Sprague-Dawley rats orally administered detergent formulations solutions ranging in pH from 10.6 to 12.2, whereas Long-Evans–strain rats given alkaline drinking water (pH 11.2 to 12.0) for 1 year had no appreciable oral mucosal changes or histologic lesions to the gastrointestinal tract.

The whole-body spasms demonstrated by 2 lizards (2/6 [33.33%]) occurred at time points immediately after transmucosal administration of euthanasia solution. The lizard that exhibited this behavior at both time points was the individual with peracute erosion of the oropharyngeal and esophageal mucosa; thus, a pain response secondary to mucosal erosion must be considered. However, it is unknown if the peracute erosion was present prior to administration of the euthanasia solution and was irritated by the alkaline pH or caused by the euthanasia solution. The lizard that exhibited these behaviors immediately after the second dose of euthanasia solution had no significant oral or gastrointestinal findings aside from glossal myodegeneration and atrophy, which were
considered chronic findings. Whole-body responses (specifically arched posture) were documented in leopard geckos receiving intracelomic injections of pentobarbital. That study suspected that this response was secondary to injection pain from the alkaline solution; reported histopathological findings in that study were limited to artificial changes to the heart and kidneys. The euthanasia solution product label used for this study recommends buffering to adjust pH, and evaluation of clinical pain responses using a more neutral euthanasia solution may be considered in future studies. In laboratory rats, the addition of a local anesthetic (lidocaine, bupivacaine) reduced abdominal writhing following intraperitoneal pentobarbital, which could also have potential applicability to reptile euthanasia. In the present study, oral leakage of euthanasia solution occurred in 3 lizards (3/6 [50%]), so the utilization of buffering agents or anesthetics will likely be limited by realistic administration volume at current doses. Overall, the behavioral responses to transmucosal euthanasia solution exhibited by half of the lizards and histopathologic evidence for mucosal damage in 1 individual raise concern about distress and/or pain experienced during the euthanasia process. However, other causes of these behaviors must be considered, including CNS excitatory effects of the pentobarbital-phenytoin solution. Preadministration of additional analgesic, sedative, and/or anesthetic agent(s) prior to transmucosal euthanasia application may decrease adverse behavioral reactions and alter the timeline by which death is attained. This may also decrease the need to administer a staged transmucosal protocol and reduce oral leakage by allowing the placement of euthanasia solution into the large pharyngeal region that is present in bearded dragons and should be investigated in future studies. Given the reported histologic lesions, transmucosal pentobarbital administration is not recommended for individuals where necropsy evaluation will be pursued, particularly if a lesion of the oral cavity or upper gastrointestinal tract is to be investigated.

Death, based upon cessation of a beating heart, was achieved in all lizards within 360 minutes (6 hours), with a median time of 300 minutes (5 hours). This is prolonged compared to previous studies evaluating pentobarbital administered via intracardiac or intracelomic injection in leopard geckos (cardiac arrest was within 45 minutes in the intracardiac group and within 60 minutes in the intracelomic group) but more rapid compared to pond turtles euthanized via transmucosal pentobarbital (median time to death was 1,080 minutes [18 hours]). In our study, transmucosal euthanasia solution resulted in a sedated state (median time to loss of palpebral reflex at 25 minutes) and a deeply anesthetized state (median time to loss of deep pain response at 30 minutes) within timeframes veterinarians can realistically utilize in clinical practice. However, the prolonged time to confirmation of death may be difficult to accommodate in companion animal practice if the expectation is that the reptile patient is not deceased unless myocardial contractions have ceased. Unfortunately, due to intrinsic myocardial contractility that occurs past the time of cerebral death in reptiles, waiting for the arrest of cardiac activity may not be the best indicator of reptile patient death, although it is routinely used in clinical practice. Further, it is unknown if there is continued brain activity past cessation of myocardial contractions, necessitating further work in this area. Based upon AVMA guidelines, a secondary physical method could be used to stop the heart (pithing, decapitation), or an IV or intracardiac injection of potassium chloride could be given after loss of consciousness. Given the challenges to many practitioners of IV administration in reptiles and the nature of companion animal medicine, where many of the physical methods would not be tolerated, further studies should be pursued to investigate a route and dose of euthanasia solution that may be able to induce euthanasia in a shorter timeframe.

Our data indicate that transmucosal pentobarbital administration in bearded dragons can induce clinical euthanasia within 6 hours and does not require that the patient is kept within their preferred optimum temperature zone. Given that 1 lizard had evidence of acute mucosal changes and 4 showed behaviors concerning for pain and distress, this protocol cannot be recommended without premedication of analgesic and/or anesthetic medications. Although limited by a small sample size, our study provides useful information for euthanizing bearded dragons that does not require IV administration. Future studies should evaluate this method in other species of reptiles, the addition of analgesic and/or anesthetic pre-euthanasia medications, and additional doses of pentobarbital to optimize euthanasia techniques.

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