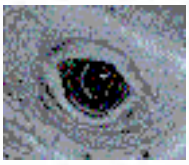

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The Use of Hormone Antagonists to Inhibit Reproduction in the Lizard, *Eublepharis macularis*

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ABSTRACT
With the increase in popularity of reptiles as personal pets, there is an increasing desire to inhibit reproduction and the associated, and often times undesirable, effects. In an attempt to develop a therapeutic treatment to induce a temporary inhibition of reproduction, we implanted time-released pellets of tamoxifen, an estrogen binding inhibitor, or indomethacin, a prostaglandin synthesis inhibitor, intracoelomically into leopard geckos, Eublepharis macularius. All females treated with either tamoxifen or indomethacin (n = 6 for each group) at the onset of the reproductive season failed to show any follicular yolk deposition for the duration of the 60-day life span of the pellets, whereas 15 of indomethacin treated females were completely inhibited for the entire reproductive season, but 50% of the females developed a generalized edema. Fifty percent of tamoxifen treated females showed some follicular vitellogenesis after the expiration of the pellet, but no female successfully laid a viable clutch. Treatment with tamoxifen during active vitellogenesis was ineffective at inhibiting further follicular growth or ovulation. Eight of nine tamoxifen females successfully reproduced in the year following treatment and their reproductive output (i.e., number of total clutches, number of fertile clutches) was similar to that of control females. These results suggest that tamoxifen may be a viable treatment to induce temporary reproductive inhibition in reptiles, and further study is warranted.

KEY WORDS: leopard gecko, Eublepharis macularius, tamoxifen, indomethacin, reproduction, inhibition.

INTRODUCTION

Successful reproduction of captive reptiles is oftentimes the primary goal of many herpetoculturists, with the emphasis on maximizing reproductive frequency and output. However, reptiles are becoming more common as household pets, and, in these

situations, reproduction may be undesirable and complications associated with reproductive activity can be life threatening (see Lloyd. 1990, DeNardo, 1996, for review of reptile dystocia). For most reptiles, not co-housing males and females can avoid reproduction. However, in some circumstances, it is neither feasible nor desirable to keep animals individually. Additionally, in some species, such as the commonly kept green iguana, Iguana iguana, females will become reproductively active even in the absence of a male (Whittier and Crews, 1986, DeNardo, 1996).

While a natural phenomenon, reproductive activity (e.g., vitellogenesis, ovulation, and oviposition) is associated with physiological and behavioral changes that owners would prefer not to experience. Such changes include extended inappetence, weight loss, muscle atrophy, and increased activity (Lloyd, 1990, DeNardo 1996, Stahl, 1997). Furthermore, because the captive environment usually does not duplicate the complexity of the natural nesting environment, gravid females can experience dystocia because of this unwanted and unproductive reproductive activity. While the green iguana is the most popular lizard that experiences "spontaneous" reproductive cycling, such cycling is also exhibited by other reptile taxa (e.g., chameleons, tortoises) and it has resulted in clinical disease and even death in captive individuals of these species also (DeNardo. 1996).

To date, treatment options are limited with owners either enduring this annual process along with the associated potential health risk or having their pet ovariectomized, which is costly and irreversible. However, current knowledge of the endocrine control of the reproductive cycle of reptiles makes it theoretically possible to inhibit hormones critical to vitellogenesis and ovulation and therefore inhibit reproductive activity inexpensively and temporarily, thereby maintaining the potential for reproduction in the future.

A suite of hormones regulates the female reproductive system. Estradiol is the primary stimulus for yolk synthesis in the reptilian liver (Yaron and Widzer, 1978, Ho, et al, 1982), while prostaglandins appear to play a vital role in follicular development and ovulation. In the Italian wall lizard, Podarcis sicula, prostaglandin E (PGE) is important in starting and sustaining oocyte vitellogenic development (via mediation of estradiol) and prostaglandin F_{2a} (PGF_{2a}) induces ovulation (via mediation of progesterone) (Gobetti, et al, 1993). While not the only hormones involved, both estrogens and prostaglandins have substantial roles in regulating female reproductive activity, and, therefore, chemical inhibition of either of these hormones should have a dramatic effect on reproduction.

Tamoxifen is a widely used inhibitor of estrogen binding and has been shown to inhibit estrogen-induced vitellogenesis in birds (Gschwendt, et al, 1982), amphibians (Riegel, et al, 1986), and fish (Le Menn, et al, 1980). It is widely used in studies to ascertain the estrogenic potency of chemicals (Pelissero, et al, 1993). Indomethacin has been shown to inhibit ovarian prostaglandin synthesis in mammals (Espy, et al, 1982), birds (Shimada, et al, 1986), fish (Goetz, 1983), and reptiles (Guillette, et al, 1990). In lizards, indomethacin delays both parturition (Guillette, et al, 1991) and follicle stimulating hormone-induced ovulation (Jones, et al, 1990), but its effect on follicular growth is unknown. Due to their effective inhibition of key reproductive hormones, we hypothesized that slow-release implants of tamoxifen and indomethacin would inhibit reproductive activity in lizards

effectively and reversibly, if so, these chemical treatments could provide a clinically valuable alternative to the limited options currently available for assuring reproductive inactivity (e.g., ovariosalpingectomy).

METHODS

Study animal and general care

Captive-born colonies (12 male and 30 female) of leopard geckos were used for this study. Leopard geckos are large (220 mm) terrestrial geckos (family Eublepharidae) native to Asia (Henkel and Schmidt, 1995). They are easily maintained and readily breed in captivity with females laying multiple clutches over several months following spring emergence.

Animals were individually housed at Arizona State University in 51 cm x 20 cm translucent plastic drawer cages (Freedom Breeder, Danville, CA). During the majority of the year, room temperature was 25 ± 1° C (77 ± 1.8° F) and sub-surface supplemental heat was provided using Mylar heat tape (Flex Watt, West Wareham, MA), thereby providing the lizards with a thermogradient of 22 - 36° C (72 - 97° F). Room lights were set to provide a 12:12 photoperiod. Paper towels were used for substrate and a deli cup containing moist vermiculite was provided as a retreat and an ovipositorium. Lizards were fed five to ten crickets dusted with calcium carbonate two to three times weekly. Amount and frequency varied depending on season. Cages were misted twice weekly.

In January, the animal room temperature was dropped to 15° C (59° F) and the supplemental heat source and lights were turned off for approximately one month. This cooling period leads to a stimulation of reproductive activity upon the return to warmer temperatures.

Experimental Design Phase 1: Inhibition of the Onset of Reproductive Activity

The goal of the first phase of the experiment was to determine the efficacy of tamoxifen and indomethacin at preventing the onset of reproductive activity. Ten days after emergence from the cooling period, six female geckos were randomly assigned to each of two treatment groups, while the remaining 18 female geckos served as controls. One treatment group (tamoxifen group) received a 60-day time-release pellet (Innovative Research of America, Sarasota, FL) containing 5 mg tamoxifen while a second treatment group (indomethacin group) received a similar implant containing 5 mg indomethacin. Pellets were implanted intracoelomically through an approximately 3 mm flank incision while under isoflurane anesthesia. The incision was closed with an everting mattress suture of 4-0 Vicryl (Ethicon, Somerville, NJ). Control animals underwent a similar surgery, but did not receive a pellet.

Four days after implantation, we began introducing a male into the female cages. Each of the 12 males was haphazardly rotated through the colony, spending 24 to 48 hr with a given female before rotating to another female. Females were exposed to at least one male every week for the duration of the reproductive season. Females were weighed and

visually inspected for reproductive activity weekly. Both follicles and eggs are visible and distinguishable through the semi-transparent abdominal skin. When follicles were present, their diameters were measured using calipers. Gravid females were checked daily for the presence of eggs. Upon oviposition, date of oviposition, female mass, egg number, egg mass, and egg quality were recorded. Data was compared between groups using unpaired T-test (StatView, SAS Institute, Cary, NC).

Experimental Design Phase 2: Inhibition of Active Vitellogenesis

The goal of the second phase of the experiment was to determine the effectiveness of the therapeutic agents at interrupting active vitellogenesis. At 120 days post-treatment (two times the projected life span of the pellets), control females with follicles were alternately subjected to either implantation with a pellet similar to those used in phase I or a second control surgery. After implantation, females were monitored and data collected as described in Phase 1.

Experimental Design Phase 3: Reversibility of Reproductive Inhibition

The goal of the third phase of the experiment was to determine whether any reproductive inhibition caused by the implanted pellets was reversible (e.g., would females that were reproductively inhibited one year successfully reproduce the next year). After year one, females were similarly cooled the following January to again stimulate reproductive activity. Upon emergence, females were not manipulated, but simply maintained as in the previous year. Data collection and analysis followed that of the previous phases of the experiment.

RESULTS

Both tamoxifen and indomethacin successfully inhibited follicular yolk deposition ($P < 0.001$ for both treatments). All six tamoxifen treated and all six indomethacin treated females showed no identifiable vitellogenic activity during the first 60 days after implantation (the projected duration of the implanted pellets). Fourteen of 18 control females initiated vitellogenic activity within this time period with the average onset occurring 22 days (range 3-60 days) post-surgery. Beyond the 60-day period, three of the six tamoxifen treated females showed onset of some vitellogenic activity (range 81-165 days post implantation), but no female laid a viable clutch. None of the six indomethacin treated females showed any vitellogenic activity for the entire reproductive season. One of the four control females that had no vitellogenic activity in the first 60 days initiated vitellogenic activity on day 102. While tamoxifen treatment caused no apparent clinical or behavioral abnormalities, three of six indomethacin treated females developed moderate to severe chronic generalized edema that was self-limiting. Due to this complication, phase two and phase three of the experiment was conducted only with tamoxifen treatment.

Beginning 120 days post-implantation, only seven control females were identified with large follicles. Four of these females received a tamoxifen pellet and three received a

sham surgery. Tamoxifen was ineffective at arresting follicular development as three of the four tamoxifen treated females and two of the three control females proceeded to ovulate and oviposit eggs resulting from the follicles that were present at the time of surgery (P = 0.846).

One of the ten tamoxifen treated females died during the following cooling period leaving nine tamoxifen treated females for phase three of the experiment. Since this death occurred during the cooling period, the death was not discovered soon enough to permit an effective necropsy. However, because of the timing of the death was more than six months beyond the expected life of the implant, it is unlikely that this death was related to the treatment. Five control females from the first year were randomly selected to remain as controls for phase three. No difference between the groups was detectable, as eight of the nine tamoxifen treated females successfully laid at least one clutch (mean = 3.3 total clutches, 2.6 fertile clutches) during the second season, while all five control females laid at least one clutch (mean = 3.0 total clutches, 2.2 fertile clutches; P = .790 and P = .708 for total clutches and fertile clutches, respectively).

DISCUSSION

While indomethacin was effective at completely inhibiting follicular yolk deposition during the entire reproductive season, the generalized edema which occurred in 50% of the females indicate that it would not be clinically valuable as an inhibitor of reproduction. Since prostaglandins have a broad range of biological activities and indomethacin is a generalized inhibitor of prostaglandin synthesis, the occurrence of a side effect is not surprising. When used as a non-steroidal anti-inflammatory agent in humans, indomethacin has a low (<1%) incidence of edema (Arky, 1999). While non-lethal and self-limiting, the generalized edema seen in indomethacin treated E. macularius was severe enough to discourage its clinical use.

The clinical value of tamoxifen remains in question, but results reported here make it a promising therapeutic option for the temporary inhibition of reproduction. Tamoxifen completely inhibited vitellogenesis during the pellet life span and inhibited successful reproduction for the entire active season.

Furthermore, females effectively treated with tamoxifen one year had normal reproductive effort the following year when they were untreated. Combined, these results suggest that tamoxifen could be used clinically to provide effective but reversible reproductive inhibition.

The failure of tamoxifen to arrest late-stage follicular development is understandable. As a blocker of estrogen binding, tamoxifen should be effective at inhibiting those reproductive processes stimulated by circulating estrogen. These include female receptivity, yolk synthesis by the liver, and yolk deposition into the follicles. It is unknown whether estrogen has any role in follicular maturation and ovulation, and our results suggest that it does not.

Based on our data, tamoxifen would be an effective inhibitor of reproduction only when given prior to or during early follicular growth. In species that respond predictably to seasonal changes (e.g., reduced ambient temperatures), the timing of tamoxifen treatment could be easily determined. However, tamoxifen may be of little value in individuals or species where the onset of reproductive activity is variable.

While, like ovariectomy, tamoxifen implants require anesthesia and entrance into the coelomic cavity, the invasiveness of the procedures are quite different. Ovariectomy requires a surgical plane of anesthesia and extensive manipulation of internal tissues. Contrarily, the implantation procedure can be done using a local anesthetic, a 3 mm incision, simple insertion of the pellet into the coelomic cavity, and a single stitch for wound closure. Total surgical time for pellet implant is approximately two minutes. Therefore, the use of tamoxifen implants potentially provides a less invasive and less time-consuming alternative to ovariectomy. While tamoxifen treatment is not the obvious clinical solution for all cases where the goal is reproductive inhibition, it may be a valuable option in many cases, especially those where only temporary inhibition is desired. Further studies, including trials in more clinically relevant species (e.g., green iguanas, tortoises) are justified.

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