Iophenoxic acid as an oral biomarker in white-tailed deer

Lisa M. White, David P. Huetter, Samuel B. Linhart, Peter J. Savarie, and Michael D. Van Brackle

Abstract We determined the dose and duration of iophenoxic acid (IA; α-ethyl-3-hydroxy-2,4,6-triiodobenzenepropanoic acid) as a biomarker for white-tailed deer *(Odocoileus virginianus)*. Ten deer were treated with IA to determine necessary dose of IA as a biomarker. Deer treated with 5 mg IA/kg had elevated iodine concentrations for 3 weeks after dosing.

Key words biomarkers, iophenoxic acid, *Odocoileus virginianus*, protein-bound iodine, white-tailed deer

Biomarkers are used in monitoring animal movements, to determine bait acceptance by target and non-target animals (Savarie et al. 1992), and to evaluate the efficacy of oral vaccine programs. For example, Hable et al. (1992) used tetracycline and rhodamine B to identify the percent of free-ranging raccoons (*Procyon lotor*) ingesting bait for potential delivery of an oral rabies vaccine. Tetracycline and iophenoxic acid (IA) have been used to evaluate the potential of oral vaccines for disease control in wild swine (*Sus scrofa*; Fletcher et al. 1990).

Iophenoxic acid (α-ethyl-3-hydroxy-2,4,6-triiodobenzenepropanoic acid) has been used as an oral biomarker in several species of wildlife (Larson et al. 1981, Baer et al. 1985, Hadidian et al. 1989, Fletcher et al. 1990, Eason and Batcheler 1991, Hable et al. 1992). It binds with serum proteins and is detected by elevated protein-bound iodine in the blood serum (Larson et al. 1981). A major advantage of IA is that it can be detected from a blood sample without killing the animal (Larson et al. 1981).

Our objective was to evaluate IA as an oral biomarker for white-tailed deer (*Odocoileus virginianus*). We wanted to determine the concentration of IA necessary to mark white-tailed deer and the duration of elevated iodine in the blood serum of deer orally treated with this biomarker.

Materials and methods

The study was conducted at The University of Georgia (UGA) deer research facility at Whitehall Forest in Athens, Georgia. All deer were housed in 3- x 6-m pens with food and water available *ad libitum*. Deer used in this study were handled according to protocols approved by the UGA Institutional Animal Care and Use Committee.

Determination of IA dose

In April 1992, 10 captive deer (1–6 years old; 7 females, 3 males) were immobilized and anesthetized with a mixture of xylazine hydrochloride (2–3 mg/kg; Mobay Corp., Shawnee, Kans.) and ketamine hydrochloride (12–18 mg/kg; Aveco, Inc., Fort Dodge, Ia.) delivered remotely via intramuscular injection from a blowdart. Blood samples were taken from the jugular vein to determine pretreatment baseline iodine concentrations. The deer were treated with either 0, 6, 60, or 300 mg of IA (2–3 deer/treatment group). The IA was suspended in propylene glycol (50 mg IA/ml) and administered via esophageal intubation. Sedation was reversed by yohimbine hydrochloride (0.5–0.8 mg/kg, Mech et al. 1985); Sigma Chemical Co., St. Louis, Mo.). Half of the dose was given intramuscularly, half intravenously.

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Table 1. Mean serum iodine concentration, body weight, and effective dose of iophenoxic acid (IA) for captive white-tailed deer given 0, 6, 60, and 300 mg iophenoxic acid (IA).

Dose (mg)	*n*	Body weight (kg) $\bar{x}$ (SE)	Mean dose (mg/kg body weight)	Iodine conc. 6 days post-treatment (μg/100ml) $\bar{x}$ (SE)[a]
0	2	34.5 (9.5)	0	10.7 (1.4) A
6	2	53.0 (9.0)	0.1	21.5 (2.5) A
60	3	48.3 (5.4)	1.2	139.0 (36.8) A
300	3	53.7 (5.8)	5.6	513.3 (63.9) B

[a] Means with the same letter in the same column are not different from deer treated with 0 mg IA (control). Significance determined by linear contrasts.

Six days after treatment, deer were immobilized, bled by cardiac puncture, and euthanized with T-61 Euthanasia Solution® (0.55 ml/kg; Taylor Pharmacal Co., Decatur, Ill.). The deer were weighed to the nearest kg and weights were used to calculate exact dosages of IA (Table 1).

Duration of IA treatment

Five captive females 1-5.5 years old, weighing 26.6-36.4 kg, were used to test the duration of IA treatment. Two does were pregnant at the beginning of the study, and 2 other does were bred after the study began. The fifth doe was not bred.

Each deer was immobilized, anesthetized as before, and weighed to the nearest kg. We took a blood sample from the jugular vein to determine baseline iodine concentrations. Iophenoxic acid was orally administered via intubation at a rate of 5 mg/kg of body weight (dose determined from previous study) and followed by 20 ml of propylene glycol to flush the tube. At 1, 3, 5, 7, 9 and 11 weeks post-treatment, each deer was immobilized and a blood sample was taken from the jugular vein. Immobilization was reversed as before.

Measurement and analysis

Blood samples were centrifuged and serum was sent to a commercial laboratory (SmithKline Beecham, Clinical Laboratories, 7600 Tyrone Ave., Van Nuys, Calif.) for total iodine analysis. All data were analyzed using SAS (SAS Inst., Inc., Cary, N.C.). Analysis of variance (ANOVA) was used to detect differences in iodine concentrations pre- and post-treatment for the 3 IA doses used. Linear contrasts were used to compare each dose to the control. ANOVA was also used to determine differences in iodine concentration among weeks. The concentration of iodine for each time point was compared to pre-treatment iodine using linear contrasts.

Results

Mean pre-treatment iodine concentration was 14.8 μg/100 ml serum in the 10 deer used to determine the effective dose of IA. Body weights ranged from 25-64 kg. Following treatment, only the group given 300 mg IA (5.6 mg IA/kg body weight) showed iodine levels above ($P = 0.0003$) the control (Table 1). Therefore, 5 mg IA/kg body weight was chosen to measure IA duration in the next study.

In the 5 deer treated with 5 mg IA/kg body weight, iodine concentration differed during the 11 weeks of the study ($F = 27.42$; 6, 28 df; $P = 0.0001$). Mean pre-treatment iodine concentration was 23.2 μg/100 ml serum (Table 2). Mean iodine concentrations rose to 516 μg/100 ml 1 week after dosing and declined at 3 weeks to 176.2 μg/100 ml. By week 5, iodine concentrations were not different from pre-treatment concentrations and remained steady for the duration of the study (Table 2).

Discussion

The concentration of iodine in serum depends upon both IA dose level and species (Perlman et al. 1955, Larson et al. 1981, Baer et al. 1985). Elevated iodine occurred 8 weeks after IA treatment in 5 species of mammalian carnivores (Larson et al. 1981). Dogs have a longer retention of IA than do foxes (*Vulpes* sp., Baer et al. 1985). However, IA did not increase iodine in rock squirrels (*Spermophilus variegatus*) or 9 species of birds (Larson et al. 1981).

The only other ruminant examined for IA retention time was the feral goat (*Capra hircus*) in New Zealand (Eason and Batcheler 1991). Oral treatment with 1.5 mg/kg was sufficient to mark the goats for at least 3 months. The level of iodine could be used to differentiate between 2 doses administered on

Table 2. Mean iodine concentrations (μg/100ml) in blood samples from 5 captive white-tailed deer treated with 5 mg/kg iophenoxic acid (IA).

Week	Concentration (μg/100ml) $\bar{x}$	SE	Range	P[a]
0	23.2	2.42	14.0–28.0	—
1	516.0	64.39	370.0–750.0	0.0001
3	176.2	59.95	73.0–400.0	0.0042
5	71.4	24.89	34.0–170.0	0.3340
7	31.4	5.28	22.0–51.0	0.8684
9	23.6	3.33	14.0–33.0	0.9935
11	25.0	3.05	15.0–31.0	0.9710

[a] Linear contrast comparison between designated time point and Week 0.

treated leaves. In our study, 1.2 mg IA/kg (60 mg IA dose group) was not different from the controls. In the deer treated with 5 mg IA/kg, iodine concentrations remained higher than pre-treatment concentrations for only 3 weeks after dosing.

Albumin tightly binds IA in blood plasma. However, Astwood (1957) reported albumin is metabolized 30–40 times faster than IA. Long persistence of IA in some species suggests the compound is capable of associating with new albumin when the old is metabolized (Astwood 1957). Excretion of IA is mainly through urine in rats, dogs, and humans (Perlman et al. 1955). However, excretion rate the first 48 hours is much higher for rats (Perlman et al. 1955). Perhaps the short duration of elevated iodine in white-tailed deer was due to different metabolism in this species. This short persistence of the marker may be beneficial. For example, treated deer could be monitored for 3 weeks and used for subsequent studies.

The safety of IA for marked deer and potential risk of IA transfer up the food chain are also important considerations when evaluating a potential biomarker. Iophenoxic acid was originally used for x-ray visualization of the gallbladder in humans and had no side effects in laboratory animals (Margolin et al. 1953). Acute oral toxicity of IA as measured by LD_{50} in mice and rats is 1,850 and >5,000 mg/kg, respectively (Margolin et al. 1953). Even when administered intravenously, the LD_{50} in mice is 374 mg/kg (Hoppe et al. 1970). Toxicity of IA only occurs with doses which are magnitudes greater than the levels needed to mark animals. In our study, iodine concentrations quickly returned to baseline. Therefore, IA should be safe for white-tailed deer.

Iophenoxic acid (5 mg/kg) appeared safe and effective for marking white-tailed deer for at least 3 weeks after oral treatment. This period should be sufficient to gather many types of useful data on this species such as movement patterns during peak rut or fawning, bait acceptance, and efficacy of oral vaccine programs.

Future research with oral biomarkers should include work on bait formulations for maximum acceptance by deer. Evaluation could include administration of IA as a suspension on preferred deer browse such as Japanese honeysuckle (*Lonicera japonica*). Eason and Batcheler (1991) successfully treated feral goats with IA-treated mahoe (*Hibiscus* sp.) leaves. There have been no reports of IA given intramuscularly to mark wildlife. Darting deer with IA may be possible, but intramuscular treatment of IA should be evaluated first.

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Literature cited

Astwood, E. B. 1957. Occurrence in the sera of certain patients of large amounts of a newly isolated iodine compound. Trans. Assoc. Am. Phys. 70:183–191.

Baer, G. M., J. H. Shaddock, D. J. Hayes, and P. J. Savarie. 1985. Iophenoxic acid as a serum marker in carnivores. J. Wildl. Manage. 49:49–51.

Eason, C. T., and D. Batcheler. 1991. Iophenoxic and iopanoic acid as bait markers for feral goats. Wildl. Res. 18:85–90.

Fletcher, W. O., T. E. Creekmore, M. S. Smith, and V. F. Nettles. 1990. A field trial to determine the feasibility of delivering oral vaccines to wild swine. J. Wildl. Dis. 26:502–510.

Hable, C. P., A. N. Hamir, D. E. Snyder, R. Joyner, J. French, V. Nettles, C. Hanlon, and C. E. Rupprecht. 1992. Prerequisites for oral immunization of free-ranging raccoons (*Procyon lotor*) with a recombinant rabies virus vaccine: study site ecology and bait system development. J. Wildl. Dis. 28:64–79.

Hadidian, J., S. R. Jenkins, D. H. Johnston, P. J. Savarie, V. F. Nettles, D. Manski, and G. M. Baer. 1989. Acceptance of simulated oral rabies vaccine baits by urban raccoons. J. Wildl. Dis. 25:1–9.

Hoppe, J. O., J. H. Ackerman, A. A. Larsen, and J. Moss. 1970. Sodium tyropanoate, a new oral cholecystographic agent. J. Med. Chem. 13:997–999.

Larson, G. E., P. J. Savarie, and I. Okuno. 1981. Iophenoxic acid and mirex for marking wild, bait-consuming animals. J. Wildl. Manage. 45:1,073–1,077.

Margolin, S., I. R. Stephens, M. T. Spoerlein, A. Makovsky, and G. B. Belloff. 1953. Experimental oral cholecystography with a new contrast medium, teridax (triiodoethionic acid). J. Am. Pharm. Assoc. 42:476–481.

Mech, L. D., G. D. DelGiudice, P. D. Karns, and U. S. Seal. 1985. Yohimbine hydrochloride as an antagonist to xylazine hydrochloride-ketamine hydrochloride immobilization of white-tailed deer. J. Wildl. Dis. 21:405–410.

Perlman, P. L., R. E. Kosinski, and D. Sutter. 1955. Studies on the absorption and excretion of a new cholecystographic agent, teridax. J. Am. Pharm. Assoc. 44:69–74.

Savarie, P. J., B. E. Johns, and S. E. Gaddis. 1992. A review of chemical and particle-marking agents used for studying vertebrate pests. Proc. 15th Vertebr. Pest Conf. 15:252–257.

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