EFFICACY, SAFETY, AND REVERSIBILITY OF A BISDIAMINE MALE-DIRECTED ORAL CONTRACEPTIVE IN GRAY WOLVES (Canis lupus)

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Abstract: A trial of the efficacy and safety of the male oral contraceptive bisdi'amine (WIN 18,446) was conducted using 10 male gray wolves (Canis lupus) (six treated, four control). During phase I, treated wolves were fed 9 g bisdi'amine daily for 5 mo, beginning in early November 1992 before the onset of spermatogenesis. During phase II, two of the previous control males were given bisdi'amine daily, beginning in January 1994 after sperm were detected in semen, to determine whether this dosage was sufficient to suppress ongoing spermatogenesis. In addition, reversibility was evaluated in the six previously treated males. Wolves were anesthetized for electroejaculation and blood collection monthly during both phases. At the end of phase I, testicular biopsies were taken for histology. During phase I, sperm first appeared in the semen of control wolves in early December and continued through April. Sperm were seen in five of the six treated wolves, but at very low concentrations compared with controls and after cessation of treatment. All treated males were housed with females, but although at least one was seen mating, none of the females gave birth. Hematologic values remained within the normal range for domestic dogs. Serum chemistry values did not differ between treatment and control wolves, although some values fell outside the normal range for domestic dogs. During phase II, sperm were found in semen samples of all males, although at much lower numbers in those of wolves given bisdi'amine. No deleterious effects were found. These results suggest that the bisdi'amine WIN 18,446 is a safe, effective, and reversible oral contraceptive when administered daily to gray wolves. Further work is necessary to clearly establish the minimum dosage required to accomplish infertility.

Key words: Contraception, bisdi'amine, wolf, Canis lupus, WIN 18,446, fertylins.

INTRODUCTION

Because of concern for responsible gen- netic management and prevention of surplus animal production, virtually all zoos in North America use contraception. The most common contraceptive is the synthetic pro- gestin melengestrol acetate (MGA), incorporated in a silastic implant.

Research has been initiated on alternative contraceptives to provide managers of free-ranging and captive wildlife with better choices. Also, several reports have correlated long-term use of MGA in felids with uterine pathology. A review of the deleterious effects of synthetic progestins reported in domestic dogs and cats suggests that other canids and likely other carnivores such as bears, mustelids, and pinnipeds may experience the same effects. Thus, an alternative to steroid contraception is critically needed.

During studies of the toxicology of bisdi'amines, a class of amebicides, the testes of treated animals were found to be specifically affected. Oral administration to dogs, rats, guinea pigs, rhesus monkeys, and humans resulted in a completely reversible arrest of spermatogenesis. Sper- matid maturation was disrupted, but Sertoli cells, germinal epithelium, spermatogonia, and membranes of the seminiferous tu- bules were unaffected. However, no effect on the production and secretion of testo- sterone by Leydig cells was found. Unlike other agents that disrupt the germinal epithelium, bisdi'amines did not affect other highly proliferating tissues such as the
intestinal mucosa, lymph nodes, and bone marrow.

The most promising bisdiamine male oral contraceptive was WIN 18,446 (Sterling Winthrop, Rensselaer, New York 12144, USA), because it had greater antispermatogenic activity without being amebicidal. However, the drug was never marketed because of its antabuse effect (e.g., nausea, sweating) following consumption of alcoholic beverages apparently because of interference of the drug with alcohol dehydrogenase activity. Although no other side effects have been found in males, the compound cannot be given to pregnant females because of its teratogenic effects on fetuses.

Because the drug had already been tested in the domestic dog, we selected the gray wolf (Canis lupus) as a model for wild carnivores. This study was designed to test the efficacy and reversibility of WIN 18,446 as a contraceptive, with special attention to possible side effects.

MATERIALS AND METHODS

The study consisted of two phases during consecutive breeding seasons. During phase I, treatment was initiated prior to the seasonal appearance of sperm in the ejaculate. During phase II, those wolves that had received bisdiamine in phase I were left untreated and were monitored for reversal of effect. In addition, two of the phase I control males were administered bisdiamine starting in January, after initiation of seasonal sperm production had been documented, to determine whether the same dose could be effective in blocking ongoing spermatogenesis and, if so, with what latency.

Ten adult male wolves were maintained outdoors at a research facility in Minnesota. During phase I, six were treated and four served as controls. Four of the treated males were part of a social group (n = 8) that included females that was maintained in an outdoor enclosure 57 × 18.5 m (0.16 ha). The wolves were conditioned to enter individual holding kennels with drop doors located at a corner of the enclosure for handling or individual feeding. All other males were paired with females in outdoor kennels (1.8 × 3.1 m). Pairs were separated for handling by use of den boxes with drop doors at one end of each run.

Water and dry food (Mazuri Exotic Canine Diet 5M52, Purina Mills, St. Louis, Missouri 63144, USA) were available ad libitum. Powdered bisdiamine was administered daily (9 g) to each wolf individually, mixed in 0.25 kg ground meat (Nebraska Brand Feline Diet, Animal Spectrum, North Platte, Nebraska 69103, USA). Phase I treatment extended from 3 November 1992 through 1 March 1993. During phase II, the six wolves given bisdiamine in 1992–1993 were left untreated to monitor reversal, and two of the previous season controls were treated for 2 mo starting 10 January 1994. Treatment wolves weighed 38–64 kg at the start of the study, resulting in an effective dosage of 140–237 mg/kg body weight. The dose was calculated for wolves based on the minimum effective dosage for beagle dogs of 150 mg/kg body weight.

Each male was anesthetized monthly during phase I (November 1992 through May 1993) and phase II (November 1993 through May 1994) with ketamine hydrochloride (Ketaset, Fort Dodge Laboratory, Fort Dodge, Kansas 50501, USA) and xylazine (Rompun, Miles, Shawnee Mission, Kansas 64506, USA) for body weights and testis measurements. In addition, blood and semen were collected for hematology and clinical chemistries and for assessment of spermatogenesis, respectively.

Semen was collected using a standardized electroejaculation procedure. A teflon rectal probe (1.6-cm diameter) with three longitudinal stainless steel electrodes was used to administer a total of 30 electrical stimuli in three series of 10 pulses each at 3, 4, and 5 volts, with a 3-min rest period between stepwise increases. Each semen sample was examined immediately (×250) for presence of sperm cells. If sperm were
Table 1. Concentration and percent motility for sperm from control and bisdiamine-treated male wolves during phase I.

<table>
<thead>
<tr>
<th>Wolf no.</th>
<th>January</th>
<th></th>
<th>February</th>
<th></th>
<th>March</th>
<th></th>
<th>April</th>
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<tbody>
<tr>
<td></td>
<td>Concentration</td>
<td>% motility</td>
<td>Concentration</td>
<td>% motility</td>
<td>Concentration</td>
<td>% motility</td>
<td>Concentration</td>
</tr>
<tr>
<td>Bisdiamine treated</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>267</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
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<td>343</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>356</td>
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<td>0</td>
<td>0</td>
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<td>2</td>
<td>60</td>
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</table>

$\times 10^6$ sperm cells/ml.

present, motility and status (type of movement on a scale of 0 to 5: 0 = no movement, 5 = steady, rapid forward progression) were subjectively estimated. A 10-μl aliquot of semen was used to calculate sperm concentration using a hemacytometer. When sperm concentration allowed, morphology was evaluated after fixing an aliquot in 1% glutaraldehyde and examining 100–200 cells at $\times 400$.

At the end of phase I, testis wedge biopsies were taken from all wolves for histologic assessment. Samples were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 7 μm, and stained with hematoxylin and eosin (H&E). Sections were analyzed by light microscopy for morphologic evidence of damage to Leydig cells, Sertoli cells, or spermatogonia and for the degree of spermatogenesis.

Analysis of variance for repeated measures (NCSS: Number Cruncher Statistical System, J. Hintz, Kaysville, Utah 84037, USA) was used to compare effects of bisdiamine treatment on parameters of sperm analysis and on testis size.

RESULTS

During phase I, sperm were present in semen samples of all control wolves by January, with concentrations remaining high through February and declining dramatically by April (Table 1). Five of the six treated males had sperm in the ejaculate at some time during the study but at very low concentrations compared with controls (Table 1). Greater sperm concentrations were found in the heavier treated animals. Furthermore, the treated male with the highest sperm count (no. 356), one of the heaviest animals, had refused to accept drug administration on several occasions.

For January through March, sperm concentrations of control males ranged from 4 to 336 $\times 10^6$ cells/ml, whereas the range for treated males during this period was 0–9 $\times 10^6$ cells/ml. The maximum sperm concentrations of control wolves occurred in January or February (the time of female estrus), whereas those for treated males occurred in March and April. Percentages of motility and of normal morphology of sperm cells did not differ between control and treatment animals. Testis size increased during the breeding season for both groups, but the percentage of change was significantly greater ($P < 0.001$) for control males.

Histopathologic analysis confirmed spermatogenic arrest in five of six treated
wolves. Of these five wolves, two had seminiferous tubules containing spermatogonia only (nos. 343, 267), whereas three had spermatogonia and a few primary spermatocytes (nos. 355, 344, 354). The testis sample from the sixth treated wolf (no. 356) contained all stages of spermatogenesis, including mature spermatids, Sertoli cells and Leydig cells in all treated wolves were normal. Testis biopsies of control wolves showed normal spermatogenesis.

During the 4 mo of treatment in phase I, weight loss occurred in five of six treated wolves (mean ± SE loss: 7.6 ± 3.4 kg) and one of four controls (4.5 kg). These five treated wolves (3-mo-old pack members) were initially the largest animals. They were observed competing for dominance during most of the phase I breeding season, but all were at or near their initial weight by the beginning of the next breeding season.

During phase II, all wolves that had been treated with bisdiamine in the previous year produced sperm in concentrations within the phase I control range, demonstrating successful reversal (Fig. 1). However, sperm concentrations peaked in March rather than in January and February. Sperm motility and morphology were within the range typically found for wolves (control wolves in this study). Spermatogenesis was lower but not completely suppressed in the two wolves given bisdiamine starting in mid-January 1994 (Fig. 1). Their sperm concentrations ranged from 3 to 17 × 10^6 cells/ml during treatment, a significant drop from pretreatment values (P < 0.05).

Hematology values consistently fell within the range for normal domestic dogs as established by the testing laboratory. Liver enzymes (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) were mildly elevated (ALT = 8–53 IU/L; AST = 16–40 IU/L) in wolves from both groups. However, only two treated and
two control wolves had ALT values above the gray wolf range (24–96 IU/L), and all AST values were within the normal gray wolf range (32–67 IU/L). The mean (±SD) values of control wolves (ALT = 95 ± 73 IU/L; AST = 38.7 ± 7.4 IU/L) were not significantly greater than those of treated wolves (ALT = 83.8 ± 22.4 IU/L; AST = 36.6 ± 11.5 IU/L). Mean total serum bilirubin values in both treatment (0.23 ± 8.16 mg/dl) and control (0.25 ± 5.8 mg/dl) wolves were below domestic dog values (0.4–1.3 mg/dl) but not below gray wolf values (0.16–1.0 mg/dl). Total serum protein levels were below normal domestic dog values (6.3–8.1 g/dl), but mean values for treatment (5.9 ± 0.6 g/dl) and control (5.8 ± 0.8 g/dl) groups were not significantly different from each other or from the normal wolf range (5.7–7.6 g/dl). No other deleterious effects were noted.

**DISCUSSION**

Orally administered bisdiamine reversibly depressed sperm production in gray wolves with no apparent deleterious effects. However, the 9-g dose of bisdiamine appeared to be near the threshold of effectiveness; sperm, albeit at very low concentrations, were observed in semen from five of the six treated wolves. Furthermore, sperm counts were positively correlated with body weight, i.e., heavier animals received a lower dosage on a per-body weight basis and were more likely to produce sperm. Also, an individual that occasionally refused his daily dose had the highest sperm count for the treatment group. However, this particular wolf, who was the dominant male in his pack, was seen mating with the dominant female, but she did not give birth, which suggests that the male may have been infertile despite the presence of some sperm in his ejaculate. A higher dose might have assured his sterility.

Maximum sperm concentrations in the treated animals occurred in the months following female estrus. The maximum concentration for wolf no. 356 occurred in the month after cessation of treatment, which is surprising because in one study beagles showed only slight if any recovery 5 wk following discontinuation of treatment. However, spermatogenesis had been suppressed completely in the dogs, which may explain the difference; only one of the wolves was azoospermic.

The dramatic decline in sperm counts in the males for whom treatment was begun after sperm production was well under way was even more surprising. In the dog, which unlike the wolf is not a seasonal breeder and produces sperm year round, a significant reduction in spermatogenesis was not seen until after 16 wk of treatment, which suggests that spermatogenesis may be more easily arrested in the wolf than in the dog.

No deleterious effects attributable to bisdiamine treatment were found during the study. Weight loss was documented in treated males only for members of a pack that were actively and aggressively competing for dominance. The subjective impression of the caretakers was that the dissension in the group, not the treatment, was responsible for the weight loss. Treated males paired with females in kennels did not lose weight. Weight loss during the breeding season may also account for low normal total serum proteins noted in both treated and control wolves. The cause of mildly elevated ALT in both groups is unknown but was unrelated to bisdiamine treatment. Significant liver damage is unlikely because total bilirubin and AST values were normal in both groups.

This study has shown that bisdiamine WIN 18,446 is a safe and effective contraceptive for gray wolves when administered orally if treatment is initiated prior to seasonal onset of spermatogenesis.

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LITERATURE CITED


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