GnRH Immunocontraception of Male Cats

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ABSTRACT

The development of nonsurgical contraceptives for cats may facilitate population control of the species. The purpose of this study was to investigate the utility of GnRH for immunocontraception of male cats. Male cats (n=12) were divided into groups of three and were immunized once with 0 (sham), 50, 200, or 400 ì g synthetic GnRH coupled to keyhole limpet hemocyanin and combined with a mycobacterial adjuvant to enhance immunogenicity. GnRH antibody titer, serum testosterone concentration, and scrotal size were determined monthly. At 6 mo, semen was collected by electroejaculation and testes were examined histologically. GnRH antibodies were detected in all cats receiving GnRH vaccine by 1 mo post-treatment and persisted throughout the study. No dose effect of GnRH was observed as titers were not significantly different between cats treated with 50, 200, or 400 ì g GnRH (P = 0.5). Six of nine treated cats were classified as responders based on high GnRH antibody titers (>32,000). By 3 mo post-treatment, responder cats had undetectable testosterone and testicular atrophy. Nonresponder cats had GnRH titers of 4,000 to 32,000 and testosterone concentrations intermediate between responder and sham-treated cats. At 6 mo, total sperm counts were similar for sham-treated cats (3.1 ± 1.8 x 10^6 sperm) and nonresponder cats (3.4 ± 1.6 x 10^6 sperm; P = 0.7). Only one of the six responder cats produced sperm, none of which were motile. Combined testicular weights of responder cats (1.3 ± 0.1 g) were lower than sham-treated controls (5.3 ± 1.3 g; P = 0.02) and nonresponder cats (2.9 ± 0.3 g; P = 0.02). Histologic evaluation of the testes revealed that in responder cats, the interstitial cells that were present were pale and shrunken compared to the plump, polyhedral eosinophilic cells in sham-treated cats. GnRH responder cats had marked tubular atrophy with vacuolated Sertoli cells and a paucity of germ cells. Single-dose GnRH treatment resulted in testosterone concentrations and semen quality consistent with immunocastration in a majority of cats treated. Supported by NIH RR-00124.

STUDY

The development of non-surgical contraceptives for cats may facilitate population control of the species. An ideal feline non-surgical contraceptive would have a high margin of safety for treated animals and the environment, be effective in a high percentage of treated animals, have a rapid onset and long duration of activity following a single treatment, inhibit sex hormone production, be efficacious in all animals regardless of sex or age, and be simple to deliver in the field. Emerging immunocontraceptive technology provides a promising avenue for non-surgical population control.

When considering the best immunocontraceptive strategy, the highest point along the hypothalamic- pituitary- gonadal axis is a logical target. GnRH is a decapeptide produced in the cat by telencephalic and diencephalic neurons and released from the median eminence into the capillary plexus of the hypothalamus. GnRH release initiates a hormone cascade responsible for ovulation and viable sperm production, as well as undesirable nuisance behaviors such as fighting, marking, wandering, and calling, and to adverse health effects, including mammary neoplasia, pyometra, and prostatitis. Therefore, antibodies against hypothalamic GnRH prevent the normal cascade of hormone secretion that is required for gonadal regulation and gamete production. The purpose of this study was to investigate the utility of modified gonadotropin releasing hormone (GnRH) for immunocontraception of male cats.

Twelve male cats were randomly distributed into four treatment groups. Each male was immunized once with a vaccine construct containing either 0 (sham), 50, 200, or 400 ì g synthetic GnRH coupled to keyhole limpet hemocyanin and combined with a mycobacterial adjuvant to enhance immunogenicity. GnRH antibody titer, serum testosterone concentration, and scrotal size were determined monthly. At 6 months, semen was collected by electroejaculation and evaluated for concentration and motility. Males were also castrated at 6 months, and testes were examined histologically.

GnRH antibodies were detected in all cats receiving GnRH vaccine by 1 month post-treatment and persisted throughout the study. No dose effect of GnRH was observed as titers were not significantly different between cats treated with 50, 200, or 400 ì g GnRH ($P = 0.5$). Six of nine treated cats were classified as responders based on high GnRH antibody titers (>32,000). By 3 months post-treatment, responder cats had undetectable testosterone and testicular atrophy. Nonresponder cats had GnRH titers of 4,000 to 32,000 and testosterone concentrations intermediate between responder and sham-treated cats.

At 6 mo, total sperm counts were similar for sham-treated cats ($3.1 \pm 1.8 \times 10^6$ sperm) and nonresponder cats ($3.4 \pm 1.6 \times 10^6$ sperm; $P = 0.7$). Five of the six responding cats exhibited azoospermia, with the remaining male producing non-motile sperm. Combined testicular weights of responder cats (1.3 ± 0.1 g) were lower than nonresponder cats (2.9 ± 0.3 g; $P = 0.02$), and sham-treated controls (5.3 ± 1.3 g; $P = 0.02$). Evaluation of testes histology revealed that responder cats had marked tubular atrophy with vacuolated Sertoli

cells and a paucity of germ cells. In addition, the interstitial cells of responder cats were pale and shrunken when compared to the plump, polyhedral eosinophilic cells in sham-treated cats. In this pilot study, single-dose GnRH treatment resulted in testosterone concentrations and semen quality consistent with immunocastration in a majority of cats treated.

These results led to development of a full trial using the 200 ug GnRH vaccine construct. In brief, this study consisted of 24 males randomly distributed into either a treatment or sham group. Determination of GnRH antibodies, serum testosterone concentration, and semen analysis were performed monthly. Materials and methods were repeated exactly. At three months post treatment, treated males were clearly divided into 8 responder and 4 nonresponder cats. All seminal characteristics of responding cats were severely reduced when compared to sham and nonresponding cats ($P<0.05$). This study is ongoing to evaluate the duration of immunity following a single GnRH immunization.

Table 1 – GnRH antibody titer, testosterone concentration, and semen characteristics of cats 6 mo after GnRH immunization.

Cat	Treatment	GnRH antibody titer (10^3)	Serum testosterone (ì g/dL)	Total sperm count ($x10^6$)	Combined testes weight (g)
1A	Sham	0	110	6.6	7.1
1B	Sham	0	240	1.4	3.0
1C	Sham	0	240	1.2	5.7
2A	50 ì g GnRH	32	100	1.3	3.0
2B	50 ì g GnRH	256	0	0	1.2
2C	50 ì g GnRH	128	0	0.0002	1.7
3A	200 ì g GnRH	128	0	0	1.3
3B	200 ì g GnRH	32	10	2.2	2.3
3C	200 ì g GnRH	128	0	0	0.9
4A	400 ì g GnRH	128	0	0	1.5
4B	400 ì g GnRH	128	0	0	1.2
4C	400 ì g GnRH	8	110	6.6	3.3