Effect of Chlormadinone Acetate-Pellet Implantation on the Volume of Prostate, Peripheral Blood Levels of Sex Hormones and Semen Quality in the Dog

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ABSTRACT. Chlormadinone acetate (GS implant®, CMA) pellet, a synthetic luteal hormone preparation, was subcutaneously implanted at 5, 10 and 20 mg/kg in four normal male dogs ranging in age from 3 to 10 years to determine the changes in the prostatic volume, peripheral plasma levels of sex hormones and semen quality. The plasma levels of CMA, LH, testosterone (T) and 5 α -dihydrotestosterone (DHT) were measured by radioimmunoassay. The prostatic volume was measured by computed tomography. The semen was collected by digital manipulation. The pellet was removed 26 weeks after implantation. The effects of CMA pellet implantation were examined during implantation and until 22 weeks after removal. The prostatic volume was reduced to 61 $\pm$ 3 (mean $\pm$ S.E., n=4), 52 $\pm$ 5 (n=4) and 53 $\pm$ 9 (n=4)% of the preimplantation volumes in the 5, 10 and 20 mg/kg groups, respectively. The plasma CMA levels in the 10 mg/kg and 20 mg/kg groups peaked at 2 weeks, but were gradually decreased. At 22 weeks after removal of the pellet, the prostatic volume returned to 74–85% of the preimplantation volumes. The plasma LH levels tended to increase after implantation in all groups. The plasma T and DHT levels were slightly decreased in all groups. In the 10 and 20 mg/kg groups, the number of sperm and motility sperm was reduced, and the rate of abnormal sperm increased. These findings indicated that implantation of a CMA-pellet at a dose of 10 mg/kg or more decreases the prostatic volume to 1/2 the original size and retains its effect for a long time, although it depresses gonadal function. The present study suggested that CMA implantation is useful in treating benign prostatic hyperplasia.—**KEY WORDS:** canine, chlormadinone acetate pellet, prostatic hypertrophy, semen quality, sex hormone.

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The canine prostate grows larger with age. Benign prostatic hypertrophy is known to occur frequently in the dog [3]. Disturbance of defecation and urination, and discharge of bloody urine have been described as the clinical symptoms of prostatic hypertrophy occurring in the dog [6]. Since hormones secreted from the genital organs are thought to be associated with the development of prostatic hypertrophy [5, 7, 21, 25], this disease has generally been managed by gonadectomy. But, since this surgical intervention has disadvantages of involving an anesthesia-related risk and destroying reproductive potency, studies on conservative therapy by injection or oral administration of synthetic progesterone preparations have been encouraged [1, 2, 4, 15, 16, 18, 20, 24]. Treatment by injection or oral administration requires long-term repeated medication. Prostatic hypertrophy is known to recur in some cases after stopping chemotherapy [1, 2, 4]. To find a solution to these problems, a pellet of chlormadinone acetate (CMA), a synthetic progesterone preparation used as a contraceptive in female dogs [19], was implanted subcutaneously in the neck of normal male dogs to investigate the effects on the prostatic volume, plasma levels of sex hormones and semen quality.

MATERIALS AND METHODS

The animals used were 12 normal beagle dogs ranging in age from 3 to 10 years. The animals were fed on dry-type dog food twice a day and were allowed free access to fresh water. The drug used for implantation was a pellet 30 mm

in length and 5 mm in diameter containing 100 mg of CMA (GS implant®; Teikoku Hormone Mfg., Co., Ltd., Japan) with silicone as a carrier. CMA was used at a dose of 5, 10 or 20 mg/kg. Four dogs were used in each group. The dogs were anesthetized with atropine Droperidole (Droleptane®) and ketamine, and the pellet was implanted subcutaneously between the scapulae. The pellet was removed 26 weeks after implantation.

The prostatic volume was measured before and 14 and 26 weeks after implantation and 22 weeks after removal of the pellet. The animals were placed under halothane inhalation anesthesia following administration of atropine and Droperidole (Droleptane®), and the prostate was sliced by computed tomography. The cross-sectional area was calculated from the trajectories, and the prostatic volume was obtained by multiplying the cross-sectional area by slice thickness (2 mm).

Blood was collected three times (9:00, 12:00 and 15:00) per day on the days of semen sampling. To determine the plasma CMA level, blood was collected at 1, 2, 4, 8, 12 and 24 hr and 2, 3 and 5 days after implantation of the pellet and after removal. In collecting blood, heparin was used as an anticoagulant. The blood collected was centrifuged at 2,500 rpm (600G) for 15 min to separate the plasma. The plasma CMA level was measured by the GC-MS method [22] with deuteriohydrogen of CMA as an internal standard. Plasma concentrations of LH were determined by radioimmunoassay as reported previously by Nett *et al.* [17], using porcine LH (LER-778) for radioiodination and anti-porcine LH serum. Purified

canine LH (LER-1685) was used as a reference standard. The plasma levels of testosterone (T) and 5 α -dihydrotestosterone (DHT) were determined as described previously [10, 23].

Semen quality was examined at intervals of one week from 3 weeks before implantation of the pellet until 10 weeks after implantation and at intervals of 2 weeks thereafter until 22 weeks after removal. The semen sample collected by digital manipulation was divided into 3 fractions. The volume of semen, the number, motility and viability of sperm, the rate of abnormal sperm and the seminal pH were determined by the methods previously described [12]. Data on changes in CMA were analyzed by student's *t* test and compared with the dose of 5, 10 and 20 mg/kg. A value of $P < 0.05$ was considered to be significant.

RESULTS

The animals developed no abnormalities in their general condition during implantation or after removal of the CMA-pellet. The drug produced no side-effects.

The changes in prostatic volume after implantation of the pellet are shown in Fig. 1 using the preimplantation volume as an index basis of 100. In the 5 mg/kg group, the prostatic volume was decreased to $72 \pm 3\%$ (mean $\pm$ S.E., $n=4$) ($P < 0.01$) at 14 weeks and to $61 \pm 3\%$ ($P < 0.01$) at 26 weeks after the implantation. In the 10 and 20 mg/kg groups, it was decreased to 65 ± 3 and $54 \pm 8\%$ ($P < 0.01$) and to 51 ± 8 and $53 \pm 9\%$ ($P < 0.01$) at 14 and 26 weeks after the implantation, respectively. The prostatic volume remained diminished to 74–85% in all groups at 22 weeks after removal.

The changes in the plasma CMA level (mean $\pm$ S.E., $n=4$) after implantation of the pellet are shown in Fig. 2. In the 5 mg/kg group, although the plasma CMA level slightly increased within 6 weeks after implantation, there

Fig. 1. Changes in degree of contraction of prostate (%) after implantation of the pellet. The preimplantation volume was used as an index basis of 100. In Figs. 1–6, values represent the means $\pm$ S.E. and the arrows represent the time of removal of the pellet. * indicates significantly different from preimplantation (Student's *t* test). CMA was used at a dose of 5 mg/kg ($\blacktriangle$ — $\blacktriangle$) ($n=4$), 10 mg/kg ($\bigcirc$ — $\bigcirc$) ($n=4$), 20 mg/kg ($\blacksquare$ — $\blacksquare$) ($n=4$).

Fig. 2. Changes in plasma CMA levels after implantation of the pellet at a dose of 5 mg/kg ($\blacktriangle$ — $\blacktriangle$) ($n=4$), 10 mg/kg ($\bigcirc$ — $\bigcirc$) ($n=4$), 20 mg/kg ($\blacksquare$ — $\blacksquare$) ($n=4$). a indicates significantly different from the 5 mg/kg group and b significantly different from the 10 mg/kg groups (Student's *t* test).

Fig. 3. Changes in plasma concentration of LH (a), T (b) and DHT (c) levels after implantation of the pellet at a dose of 5 mg/kg ($\blacktriangle$ — $\blacktriangle$) ($n=4$), 10 mg/kg ($\bigcirc$ — $\bigcirc$) ($n=4$), 20 mg/kg ($\blacksquare$ — $\blacksquare$) ($n=4$). * indicates significantly different from preimplantation (Student's *t* test).

was no marked change during the experimental period. In the 10 and 20 mg/kg groups, the levels peaked (4.26 ± 0.45 and 8.03 ± 0.56 ng/ml, respectively) at 2 weeks but gradually decreased with time. The levels at 26 weeks were 0.74 ± 0.08 and 2.09 ± 0.40 ng/ml, respectively. The levels in the 20 mg/kg group were significantly higher than in the 5 mg/kg group, except at 14 weeks, during the experimental period ($p < 0.05$). At 2 weeks after removal of the pellet, CMA could not be detected in plasma from any of the animals.

The changes in plasma LH, T and DHT levels are shown in Fig. 3. Plasma levels of LH tended to increase slightly after implantation in the 5 and 10 mg/kg groups. Especially in the 5 mg/kg groups, the levels were significantly higher than the preoperative values between 12 weeks and 26 weeks after implantation ($P<0.05$), but returned to the preimplantation levels after removal of the pellet.

The mean ($\pm$ S.E.) levels of plasma T prior to implantation in the 5, 10 and 20 mg/kg groups were 2.19 ± 0.36 , 2.30 ± 0.55 and 2.16 ± 0.17 ng/ml, respectively. They tended to decrease to less than 2 ng/ml after implantation of the pellet. In the 10 mg/kg groups, the levels were significantly lower than the preoperative values between 4 weeks and 12 weeks after the implantations ($P<0.05$). The plasma DHT concentration in all groups tended to decrease to less than 0.3 ng/ml from 4 weeks after implantation. Especially in the 5 mg/kg groups, the levels were significantly lower than the preoperative values between 6 weeks and 30 weeks after the implantation ($P<0.05$). After removal of the pellet, the plasma T and DHT levels were increased and returned to the preimplantation levels in all groups.

The changes in semen quality are shown in Figs. 4, 5 and 6. The volume of semen tended to decrease after implantation of the pellet in all groups. This change was especially pronounced in the 10 and 20 mg/kg groups. The total number of sperm also tended to decrease in the 10 and 20 mg/kg groups. Two animals each in the 10 and 20

Fig. 4. Changes in volume of semen and the number of sperm after implantation of the pellet at a dose of 5 mg/kg ($\blacktriangle$ — $\blacktriangle$) (n=4), 10 mg/kg ($\bigcirc$ — $\bigcirc$) (n=4), 20 mg/kg ($\blacksquare$ — $\blacksquare$) (n=4).

Fig. 5. Changes in sperm motility and viability after implantation of the pellet at a dose of 5 mg/kg ($\blacktriangle$ — $\blacktriangle$) (n=4), 10 mg/kg ($\bigcirc$ — $\bigcirc$) (n=4), 20 mg/kg ($\blacksquare$ — $\blacksquare$) (n=4).

Fig. 6. Changes in the rate of abnormal sperm and seminal pH after implantation of the pellet at a dose of 5 mg/kg ($\blacktriangle$ — $\blacktriangle$) (n=4), 10 mg/kg ($\bigcirc$ — $\bigcirc$) (n=4), 20 mg/kg ($\blacksquare$ — $\blacksquare$) (n=4).

mg/kg groups developed azoospermia from 20 to 24 weeks after implantation. In 3 of these 4 animals, sperm began to appear 10 weeks after removal of the pellet. Sperm motility tended to be depressed in all groups 12 weeks after the implantation, and this change was remarkable in the 20 mg/kg group. Sperm viability was also slightly affected but no statistical differences were found among the 3 groups. The rate of abnormal sperm was not affected during implantation but increased after removal of the pellet. The reason for this is not known. A bent tail was the main aberration. Seminal pH did not change markedly following pellet implantation.

DISCUSSION

Treatment canine prostatic hypertrophy has recently been attempted with a variety of synthetic progesterone preparations instead of conventional gonadectomy [1, 2, 4, 15, 16, 18, 20, 24]. Brass *et al.* [4] found that intramuscular injection of 1–2 mg/kg of CMA resulted in the disappearance of clinical symptoms in 80.6% of dogs with prostatic hypertrophy but retreatment was required in 61%. Bamberg-Thalen and Linde-Forsberg [1] treated dogs with prostatic hypertrophy with a single subcutaneous injection of 3 mg/kg (minimum dose 50 mg per head) of progesterone and achieved improvement in clinical symptoms at 4–6 weeks in 84% of dogs, and obtained radiographic evidence of a shrunken prostate in 53%, but the clinical symptoms have frequently been found to recur. Our earlier study [11] has shown that in some dogs with prostatic hypertrophy treated with 3- or 4-weeks continuous oral administration of 2 mg/kg of CMA, retreatment was required at 6 months after the treatment, but in the groups treated with 10 and 20 mg/kg of CMA, the prostatic volume was diminished to approximately 50% at 26 weeks after implantation and remained diminished to 80% even at 22 weeks after removal. Sahara *et al.* [19] reported that a plasma CMA level above 0.7 ng/ml was required in female dogs for the suppression of estrus. The results of the present series of experiments demonstrated that a plasma CMA level of 0.74 ± 0.08 (mean $\pm$ S.E., $n=4$) ng/ml was obtained in male dogs by implanting 10 mg/kg or more of CMA, that it markedly decreased prostatic volume and that this effect lasted for a long time.

Murakoshi *et al.* [13] produced artificial prostatic hypertrophy in dogs by treating them for 4 weeks with androstenediol plus estradiol-17 β (three times per week) and oral administration of CMA (six times per week) for 21 weeks. They found a remarkable decrease in prostatic weight and atrophy of the prostatic epithelium. Murakoshi *et al.* [14] also found marked atrophy of the prostate in rats treated with a massive dose (50 mg/kg/day) of CMA as compared with the control. In this study, the plasma LH level was slightly high in the 5 and 10 mg/kg groups, but the reason for the increase is not known.

It has been reported that CMA directly inhibited the uptake of testosterone and the binding of cytosol 5 α -

DHT-receptor at the prostate cell level [8]. The indirect action of CMA was mainly due to the acceleration of the T metabolic enzyme system in the liver and the inhibition of testosterone biosynthesis in the testis [8]. Honma *et al.* [9] reported that the decrease in plasma T resulted from the inhibited biosynthesis of T in the testis. We have also previously reported that gonadal endocrine function and spermatogenesis were inhibited by oral administration of CMA [11]. The decreases in plasma T and DHT levels and the deterioration of semen, as found in the present study, indicate the suppression of testicular function due to implantation of the CMA pellet. Since the semen was found to be restored to normal after removal of the pellet, it seems possible to utilize the dogs for reproduction. Further investigation seems necessary to determine how long a single CMA-pellet implantation treatment retains its effect to maintain the diminished state of the prostate.

It is considered that CMA implantation is a useful method for treating benign prostatic hyperplasia in the dog.

REFERENCES

1. Bamberg-Thalen, B. and Linde-Forsberg, C. 1988. Treatment of canine benign prostatic hyperplasia with medroxyprogesterone acetate. *Urol. Res.* 16: 67–72.
2. Bamberg-Thalen, B. and Linde-Forsberg, C. 1992. The effects of medroxyprogesterone acetate and ethinylestradiol on hemogram, prostate testes, and semen quality in normal dogs. *J. Vet. Med. A* 39: 264–270.
3. Berry, S. J., Coffey, D. S., and Ewing, L. L. 1983. Effects of aging on prostate growth in beagles. *Am. J. Physiol.* 250: 1039–1046.
4. Brass, W., Ficus, H. J., und Jochle, W. 1971. Anti-androgen-behandlung der prostatavergrößerung beim Hund. *Kleintierpraxis* 16: 95–99.
5. Brendler, C. B., Berry, S. J., Ewing, L. L., McGullough, A. R., and Cochran, R. C. 1983. Spontaneous benign prostatic hyperplasia in the beagle. *J. Clin. Invest.* 71: 1114–1123.
6. Campbell, J. R. and Lawson, D. D. 1963. The signs of prostatic disease in the dog. *Vet. Rec.* 75: 4–7.
7. Cochran, R. C., Ewing, L. L., and Niswender. 1981. Serum levels of follicle stimulating hormone, luteinizing hormone, prolactin, testosterone, 5 α -dihydrotestosterone, 5 α -androstane-3 α , 17 β -diol, 5 α -androstane-3 β , 17 β -diol, and 17 β -estradiol from male beagles with spontaneous. *Invest. Urol.* 19: 142–147.
8. Ito, Y., Kurosawa, H., Yamanaka, H., Koya, A., Imai, K., Kosaku, N., and Shida, K. 1977. The mechanism of antiandrogenic action of chlormadinone acetate. *Jpn. J. Urol.* 68: 537–552.
9. Honma, S., Ugi, S., Iizuka, K., Kambegawa, A., and Shida, K. 1977. The effects of anti-androgenic agents on metabolism and biosynthesis of testosterone (): Testosterone metabolic regulation in the liver and the biosynthesis in the testes on rats treated with chlormadinone acetate. *Folia. Endocrinol. Jpn.* 53: 703–718.
10. Kawakami, E., Tsutsui, T., and Ogasa, A. 1990. Peripheral plasma levels of LH, testosterone, and estradiol-17 β before and after orchiopexy in unilateral cryptorchid dogs. *Jpn. J.*

- Vet. Sci.* 52: 179–181.
11. Kawakami, E., Tsutsui, T., Shimizu, M., Orima, H., Makanae, Y., Yajima, K., and Ogasa, A. 1993. Effects of oral administration of chlormadinone acetate on canine prostatic hypertrophy. *Jpn. J. Vet. Sci.* 55: 631–635.
 12. Kawakami, E., Tsutsui, T., Yamada, Y., and Yamauchi, M. 1984. Cryptorchidism in the dog: Occurrence of cryptorchidism and semen quality in the cryptorchid dog. *Jpn. J. Vet. Sci.* 46: 303–308.
 13. Murakoshi, M., Inada, R., Makino, M., Suzuki, M., Mieda, M., Honma, S., and Yamanaka, H. 1990. Effects of anti-androgen (TZP-4238) on steroid-induced canine prostatic hyperplasia. Light and electron microscope investigations. *Acta Pathol. Jpn.* 40: 871–879.
 14. Murakoshi, M., Tagawa, M., Inada, R., Shoji, M., Suzuki, M., and Watanabe, K. 1992. The effects of new steroidal anti-androgen, TZP-4238, and chlormadinone acetate on the pituitary, prostate and adrenal gland of the rat: Histopathological and immunocytochemical studies. *Endocrinol. Japon* 39: 331–340.
 15. Neri, R. O., Casmer, C., Zeman, M. V., Helder, F., and Tabachnick, I. A. 1968. Effects of antiandrogen SH714 (6-chlor- δ^6 -1, 2- α -methylen-17- α -hydroxyprogesterone acetate, cyproterone acetate) on canine prostatic hyperplasia. *Endocrinology* 82: 311–317.
 16. Neri, R. O. and Monahan, H. 1972. Effects of a novel nonsteroidal antiandrogen on canine prostatic hyperplasia. *Investigative Urol.* 10: 123–130.
 17. Nett, T. M., Akbar, A. M., Phemister, R. O., Holst, P. A., Reichert, L. E. Jr., and Niswender, G. D. 1975. Levels of LH, estradiol and progesterone in serum during the estrous cycle and pregnancy in the beagle bitch. *Proc. Soc. Exp. Biol. Med.* 148: 134–139.
 18. Olson, P. N., Wrigley, R. H., Thrall, M. A., and Husted, P. W. 1987. Disorders of the canine prostate gland: pathogenesis, diagnosis, and medical therapy. *Compend. Contin. Educ. Pract. Vet.* 9: 613–623.
 19. Sahara, K., Tsutsui, S., Naitoh, Y., and Fujikura, K. 1993. Prevention of estrus in bitches by subcutaneous implantation of chlormadinone acetate. *J. Vet. Med. Sci.* 55: 431–434.
 20. Schubert, B., Schubert, G., und Weiger, G. 1978. Zur therapie der prostatahypertrophie des Hundes mit medroxyprogesteron. *Kleintierpraxis* 23: 331–332.
 21. Schulze, H. and Barrack, E. R. 1987. Immunocytochemical localization of estrogen receptors in spontaneous and experimentally induced canine benign prostatic hyperplasia. *The Prostate* 11: 145–162.
 22. Tsukamoto, K., Honma, S., Iwamura, S., Ochiai, H., and Kawabe, Y. 1988. Determination of chlormadinone acetate and its active metabolite, 3 β -hydroxy derivative, in plasma and urine by selected ion monitoring GC/MS. *Jpn. Pharmacol. Ther.* 16: 2503–2513 (in Japanese with English summary).
 23. Tsutsui, T., Tsuji, J., Kawakami, E., Yamada, Y., Amano, T., and Yamaguchi, M. 1987. Peripheral plasma androgen levels in the male dog from birth to sexual maturity. *Jpn. J. Vet. Sci.* 49: 177–179.
 24. Tunn, T., Senge, T., Schenck, B., and Neumann, F. 1980. Effects of cyproterone acetate on experimentally induced canine prostatic hyperplasia. *Urol. Int.* 35: 125–140.
 25. Wilson, J. D. 1980. The pathogenesis of benign prostatic hyperplasia. *Am. J. Med.* 68: 745–756.