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Histopathological Studies of Subcutaneous Implantation of Chlormadinone Acetate (CMA) for Preventing Estrus in Queens

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The efficacy and clinical safety of chlormadinone acetate (CMA) in preventing estrus were assessed in queens on condition that CMA was subcutaneously implanted in siliastic rubber. Thirteen queens were divided into the following four groups according to dose of CMA-administered: group 1 (n = 3), control ; group 2 (n = 3), 2.5 mg/kg; group 3 (n = 3), 5 mg/kg; group 4 (n = 4), 20 mg/kg. The implants were left in these queens for 12 months after implantation. All control animals showed signs of estrus during the experiment, with periods of anestrus of normal duration. In contrast, estrus was completely inhibited in the CMA-treated groups. Histopathologically, the uterus from group 4 had coiled branched glands with little secretion in the endometrium. Mammary glands from the CMA-treated groups showed mild lobular development with acinar proliferation and secretion. Sections through the other organs (pituitary gland, adrenal gland, ovary, and implant site) had no distinct or consistent changes that could be related to the CMA-treated. It was concluded, therefore, subcutaneous implantation of CMA could be the good drug-delivery system for reducing changes due to the antigonadotropic and glucocorticoid-like activities and serious condition in the uterine and mammary gland due to progestagenic activity.

Key words : Chlormadinone acetate (CMA), Implantation, Queen, Estrus, Mammary, Uterus

INTRODUCTION

Progestational compounds such as megestrol acetate are widely used in the treatment of a variety of feline dermatological and behavioural disorders [13, 21]. However, a number of potentially adverse effects are associated with use of megestrol acetate in cats, including the development of benign mammary hypertrohy, endometritis, pyometra, and profound adrenocortical suppression [4, 13, 21].

Subcutaneous implantation of chlormadinone acetate (CMA) was proved to be effective in preventing estrus in bitches for long periods [29]. This efficacy was attained by long-lasting stable levels of CMA in plasma of implanted bitches [31]. The long-lasting low levels of CMA attained by the implantation method may cause different changes from those reported previously using oral administration [23, 24, 31, 32].

The present study examined CMA-related changes in queens receiving subcutaneous implantation of CMA.

MATERIALS AND METHODS

Animals

Thirteen young adult queens from the research colony at Tohoku University were determined to be clinically healthy based on physical examinations. The age of all of the animals ranged from 20 to 28 months, and their body weight ranged from 2.3 to 4.0 kg at the beginning of the experiments. The animals received dry cat food (Prescription diet, Hill's Pet Nutrition, Inc.) and water *ad libitum*.

Implantation

A mixture of 20 mg CMA (17alpha-acetoxy-6-chlorpregna-4,6-dione) and silastic silicon rubber (MDX-4-4210 Medical Grade Elastomer, Dow Corning, MI), together with a coagulant for solidification, as injected 3.3 mm-diameter plastic tube to form a 20 mm-long cylindrical pellet. The pellet was cut to

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adjust the amount of CMA per dose. The pellet containing CMA was implanted subcutaneously after the injection with xylazine (Celactal, Bayer, Tokyo), 0.15 ml/kg, intramuscularly and procaine hydrochloride (Vatalar, park, Davis), 0.12 ml/kg, intramuscularly at anestrus of the estrous cycle stage.

Experiments

Thirteen queens were divided into the following groups according to dose of CMA-administered: group 1 (n = 3), control; group 2 (n = 3), 2.5 mg/kg; group 3 (n = 3), 5 mg/kg; group 4 (n = 4), 20 mg/kg. The implants were left in these queens for 12 months after implantation.

Statistical analysis.

The data were expressed as means ± SD. Homogeneity of variance was tested by Bartlett's method, and when the assumption of homogeneity of variance was met, one-way layout analysis of variance was performed. When significant difference was observed, Dunnett's multiple comparative test was performed between the control group and the other experimental groups.

RESULTS

1. Prevention of estrus in the queens

All control animals (group 1) showed signs of estrus during the experiment, with periods of anestrus or normal duration. In contrast, estrus was completely inhibited in the CMA-treated groups (groups 2 to 4).

2. Removed implants

It was found that 6.27 ± 4.55% (group 2), 12.61 ± 2.68% (group 3) and 11.95 ± 2.11% (group 4) of the CMA remained in the implants 12 months after implantation (Table 1).

3. Histopathological findings

Ovaries from the control and the CMA-treated groups had relatively small follicles without corpora lutea. No conspicuous histopathological changes were observed in any of the CMA-treated groups.

The uterus from group 4 had coiled branched glands with little secretion in the endometrium. No histopathological evidence of an endometritis, myometritis, and pyometra was found in the uterine sections from group 4. The uteri from the other CMA-treated groups were grossly and microscopically similar to those of the controls.

Mammary glands from the CMA-treated groups showed mild lobular development with acinar proliferation and secretion.

Sections through the other organs (pituitary gland, adrenal gland, and implant site) had no distinct or consistent changes that could be related to the CMA-treated.

DISCUSSION

The preventive effect of a single implantation of CMA on estrus in queens persisted for 12 months at a dose of 2.5 mg/kg to 20 mg/kg. The long-term efficacy of CMA implantation could be explained by the sustained release of CMA from silicon rubber. This sustained release was confirmed by gradual decrease in amount of CMA remaining in the implants. The dose-dependent stable levels of CMA in plasma may also have resulted from gradual release and these would explain its efficacy over long periods at high doses [19].

Larger doses or chronic oral administration of CMA has been associated with endometritis and pyometra in bitches [1, 3, 6, 24], and queens [7, 15, 18, 22, 25, 33, 37], with diabetes mellitus or abnormal glucose tolerance tests in bitches [1, 10, 34, 35] and queens [16, 27] with weight gains and increased appetites in bitches [1, 2, 34, 36], and queens [15, 25], and with mammary secretions, enlargement, and tumors [1, 2, 23, 24, 36] and cystic mucinous hyperplasia of the gallbladder in dogs [24]. Furthermore,

Table 1 Amounts of chlormadinone acetate (CMA) remaining in the implants

Dose	Duration of implantation (month)	Remaining CMA (%)
2.5 mg/kg	12	6.27 ± 4.55
5.0 mg/kg	12	12.61 ± 2.68
20.0 mg/kg	12	11.95 ± 2.11

Value are the mean ± SD.

CMA has been reported to have glucocorticoid-like activity, to causes adrenocortical suppression like glucocorticoids in rats and dogs [24]. It is resonable to assume CMA may have similar effects in cats.

The responses expected from this experiment occurred mainly in the ovary, uterus, and mammary gland. Ovulation was inhibited, follicular development and maturation suppressed, and usual cyclic responses in the other genital organs did not occur in the present study. To proliferative changes in the endometrium would be due to progestagenic activity of CMA. This change was not remarkable. Therefore, it would support the clinical evidence that reproductive activity recurred in queens from which implants were removed [19].

In our study the mammary gland seemed more sensitive than the uterus. Mammary lobular development occurred. There have been sporadic reports on the occurrence of mammary tumors in cats treated with progestogens [12, 14, 25]. Mammary fibroadenomas but not mammary carcinomas were found to be associated with treatment with a progestogen in a retrospective study [11]. The mechanism of action of exogenous progestogens in mammary carcinogenesis is still poorly understood. Their action may be, direct or indirect. Mammary gland has been reported to have high affinity for progesterone and receptors with low affinity for progesterone which also bind glucocorticoids. Some investigators believe that progesterone bound to the high affinity receptors is directly responsible for stimulating mammary gland growth [5]. Such receptors have been demonstrated in mammary gland in the cat and dog, and other species [10, 17, 28]. In addition, progesterone may act indirectly by stimulating release of certain pituitary hormones that effect growth of mammary gland. Elevated levels of GH, but not prolactin, have been reported in beagle dogs with proliferative mammary lesions, established after long-term exposure to synthetic progestins [8, 9]. The latter mechanism does not appear valid for the cat since long-term administration of megestrol acetate to cats failed to induce overproduction of pituitary GH [26]. In the present study, the proliferative changes in the mammary gland would be due to progestagenic activity of CMA. These changes were not remarkable. Changes in the adrenal gland and pituitary gland were negligible, and this indicates that the present method did not induce glucocorticoid-like activity. In this context, measurements of plasma-ACTH or -corticosteroid levels would seem to be important to clarify this problem. Further word along this line is now in progress in our laboratory.

The known and theoretical actions of small doses of progestogens are suppression of gonadotropins release [2, 36], suppression of follicular growth directly on ovaries [2, 36], suppression of estrogen [2], and androgen effects on target organs [20, 30]. The precise mechanism by which prevention of estrus in the queens by subcutaneous implantation of CMA is not yet clear from the present investigation. Further work along this line is now in progress in our laboratory.

It was concluded, therefore, subcutaneous implantation of CMA could be the good drug-delivery system for reducing changes due to the antigonadotropic and glucocorticoid-like activities and serious condition in the uterine and mammary gland due to progestagenic activity.

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