



FULL PAPER

Internal Medicine

# Clinical assessment of testosterone analogues for urethral sphincter mechanism incompetence in ten spayed female dogs

Reo NISHI<sup>1)</sup>, Tomoki MOTEGI<sup>2)</sup>, Shingo MAEDA<sup>1)</sup>, Satoshi TAMAHARA<sup>1)</sup>, Yasuyuki MOMOI<sup>1)</sup>, Naoaki MATSUKI<sup>1)</sup> and Tomohiro YONEZAWA<sup>1)</sup>\*

<sup>1)</sup>Department of Veterinary Clinical Pathobiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

<sup>2)</sup>Veterinary Medical Center, Department of Veterinary Medical Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

ABSTRACT. Urethral sphincter mechanism incompetence (USMI) is a common cause of urinary incontinence in dogs. Although estrogen is often prescribed for the medical therapy of USMI for spayed female dogs, they are known to have limited effectiveness and potential adverse effects. In castrated male dogs with USMI, testosterone reagents have been attempted besides estrogen. In this study, the effect of testosterone drugs, mainly methyltestosterone, on spayed female dogs with USMI was retrospectively evaluated. Ten spayed female dogs with USMI were included. Diagnosis of USMI was based on the results of the dogs' medical history, clinical signs, and no abnormalities in physical examinations, urinalysis, ultrasonography, X-ray imaging, and neurological examinations. Methyltestosterone was administered at doses of 0.32-1.27 mg/kg BW p.o. semel in die (sid.) to twice a week. Nine of the ten dogs had good or excellent responses 2 to 4 weeks after the start of treatment. The minimum effective dose was 0.32 mg/kg/day. Although no severe adverse symptoms occurred in any dog, a mild increase in alanine aminotransferase was temporally observed at doses of 1.0 and 1.1 mg/kg/day in the two dogs. After dose reduction or withdrawal, two of eight dogs had recurrence of urinary incontinence. Resumption of testosterone treatment clearly improved the symptoms in the two dogs. These results indicate that testosterone reagents might be an option for treating USMI in spayed female dogs as well.

**KEY WORDS:** female dog, sex steroid, testosterone, Urethral sphincter mechanism incompetence (USMI), urinary incontinence,

Urethral sphincter mechanism incompetence (USMI), earlier known as hormone-responsive incontinence, is a common cause of urinary incontinence in dogs. Based on etiological data, USMI occurs in 5% to 20% of spayed female dogs [2–4, 10, 12]. Although the mechanism for the development of USMI has not been completely elucidated yet, it could be attributed to low urethral closure pressure along with a decrease in sex steroid use [16, 23]. In general, phenylpropanolamine (PPA) and/or estrogen analogues are prescribed for the medical therapy of USMI for spayed female dogs [2–4, 10, 12]. However, it is difficult to commonly use PPA in Japan because this drug is not provided by manufacturers in this country. It is also known that the response rate of estrogens is not very high (approximately 18–65%) [14, 23]. Moreover, estrogen treatment should be carefully considered because it can have potential adverse effects, such as bone marrow suppression and myelotoxic effects [13, 14, 20]. In castrated male dogs with USMI, testosterone reagents have been attempted in addition to estrogen [1, 5, 9, 19]. Although testosterone is also known to have other potential adverse effects, no side effects have been reported. Therefore, in this study, the clinical efficacy and adverse effects of testosterone analogues on spayed female dogs with USMI were investigated.

# MATERIALS AND METHODS

### Case selection

The cases were retrospectively identified by examining the medical records of the University of Tokyo-Veterinary Medical Center (UT-VMC) covering the period between 2013 and 2019. The diagnostic protocol, inclusion criteria, and treatment methods of methyltestosterone were based on previous reports [1, 19]. Signalment, symptom history related to urinary incontinence, results

<sup>©2021</sup> The Japanese Society of Veterinary Science



This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: https://creativecommons.org/licenses/by-nc-nd/4.0/)

83(2): 274–279, 2021 doi: 10.1292/jvms.20-0515

J. Vet. Med. Sci.

Received: 28 August 2020 Accepted: 5 December 2020 Advanced Epub: 14 January 2021

<sup>\*</sup>Correspondence to: Yonezawa, T.: ayone@mail.ecc.u-tokyo.ac.jp

of urinalysis, and imaging studies were recorded. USMI was holistically diagnosed based on the results of the dogs' medical history, clinical signs of involuntary leakage of urine noted by the owner, and no abnormalities in physical examinations, urinalysis, ultrasonography, X-ray imaging, and neurological examinations. Dogs were excluded when they had any other causes of urinary incontinence, such as bladder or urethral calculi, urinary tract neoplasm, urinary tract infections, ectopic ureters, and neurologic dysfunction. Urethral pressure profilometry was not performed in any of the dogs and was not used as an inclusion criterion. After they were diagnosed with USMI, the dogs were treated with methyltestosterone (Enarmon tablet 25 mg, Aska Pharmaceutical Co., Ltd., Tokyo, Japan).

## Data collection

Information about the signalments and other treatments initiated for urinary incontinence, treatment doses of testosterones, dosing interval, clinical responses, and any adverse effects were collected from the dogs' medical records. When the patient did not return to UT-VMC before the discontinuation of methyltestosterone treatment or improvement of clinical signs, the owners and the current vets were asked to complete a telephone questionnaire.

#### Follow-up questionnaire

A follow-up questionnaire was also designed based on the study by Palme *et al.* [19]. Owners were asked to report any adverse effects noted during the administration of testosterone as well as time to clinical remission and length of response to it, if noted. Additionally, owners were asked to score their satisfaction with their dog's response to the drug from 1 to 4, by (1) indicating complete dissatisfaction due to no response (poor), (2) indicating only slight response (fair), (3) indicating that the animal was now continent most of the time (good), and (4) indicating full satisfaction due to complete continence (excellent).

## RESULTS

Ten spayed female dogs were included in the present study, as shown in Table 1. Continuous and intermittent dripping occurred in four and three dogs, respectively, while intermittent dripping only during recumbency occurred in three dogs. The median age of sterilization for these ten dogs was 8 months (range: 6 months to 5 years) and that of the first clinical signs of urinary incontinence was 3 years 8 months (range: 1 year 11 months to 16 years 11 months). Case G was previously treated with oxybutynin hydrochloride and bethanechol chloride for 1 month, but these drugs were not effective and were discontinued 2 weeks before she came to UT-VMC.

In Table 2, the doses and responses to methyltestosterone are shown. Note that the order of the cases was rearranged from Table 1 according to their response. The median time from onset to start of testosterone treatment was 17 weeks (range: 2 weeks to 11 months). The median of a single dosage was 0.9 mg/kg (range: 0.32–1.27 mg/kg) p.o. as the first treatment. While eight of them received a dose of once a day, two of them were treated every other day (*eod.*) or twice a week (Cases E and I). No dogs received any other concurrent medications. The median of the first follow-up interview date was three weeks later (range: 2–4 weeks). One of them indicated a poor response, and the treatment was discontinued upon the owner's request (Case D). Nine out of ten owners reported a partial or complete improvement of their dogs at that time. Moreover, all these nine dogs showed excellent responses from three to 18 weeks after the start of the treatment.

When the symptoms disappeared completely, five of the nine dogs (cases C, H, A, G, and E) stopped the prescription at once and had no recurrence of symptoms or adverse effects during the observation period. In nine of the ten dogs except for Case I, the plasma levels of alanine aminotransferase (ALT), alkaline phosphatase, blood urea nitrogen, and creatinine levels were investigated on the first follow-up day. Seven out of these nine dogs did not have any adverse effects (cases A, C, D, E, F, G, and H). In Case J, methyltestosterone treatment was temporally suspended from the first follow-up day because the ALT level increased to 306 U/l. Four weeks after the suspension, the ALT level improved to the reference range. Then, the same treatment was resumed. Since the symptoms completely disappeared two weeks after resumption, the frequency was reduced to *eod*. for seven weeks, and then

Dog	Breed	Type of incontinence	Age to be spayed (year)	Age at onset (year)	Previous treatment	Response to previous treatment
А	Border Collie	Continuous dripping	0.6	1	None	-
В	Labrador Retriever	Continuous dripping	1	6	None	-
С	Toy Poodle	Continuous dripping	0.5	7	None	-
D	Shiba	Continuous dripping	5	12	None	-
Е	Bichon Frise	Intermittent	1	2	None	-
F	French Bulldog	Intermittent	0.7	2	None	-
G	Mixed	Intermittent	Unknown	3	Oxybutynin, Bethanechol	Poor
Н	Jack Russell Terrier	Intermittent during recumbence	0.5	2	None	-
Ι	Samoyed	Intermittent during recumbence	0.7	3	None	-
J	Bichon Frise	Intermittent during recumbence	0.5	16	None	-

Table 1. Patient information at the start of methyltestosterone treatment

The Journal of Veterinary Medical Science the prescription was withdrawn. No further symptoms or strong ALT elevation was observed. In Case B and Case F, the treatment frequency was reduced from semel in die (*sid.*) to twice a week or *eod.*, when the symptoms completely disappeared seven and four weeks after the start of treatment, respectively. However, these two cases had recurrence of urinary incontinence one-two weeks after reduction. Then, the frequency was returned to *sid.* in Case B. In Case F, testosterone enanthate (Enarmon Depot, Aska Pharmaceutical), a testosterone ester drug, was used instead of methyltestosterone. It was administered intramuscularly every three weeks. Case F recovered completely around two weeks after rearrangement. The dog continued the medication with no recurrence during the observation period. In Case I, methyltestosterone was administered twice a week from the start. While she had an excellent response 17 weeks after the first prescription, she continued the same treatment.

## DISCUSSION

In this study, we collected ten case reports to evaluate the clinical efficacy and adverse effects of testosterone on USMI female dogs. As described above, the common medical therapies for USMI in dogs are PPA and/or estrogens. The response rate of estrogens in spayed female dogs has been reported to be 18–65%, while that of PPA has been as high as 85–90% [3, 14, 17]. In previous reports, testosterone improved the symptoms of urinary incontinence in three of eight and one of five castrated male dogs with PPA/estrogens-resistant USMI [1, 19]. In this study, methyltestosterone administration clearly improved the symptoms in nine of the ten spayed female dogs with USMI. This result apparently shows that the efficiency of testosterone could be better in spayed females than in castrated males. However, PPA or estrogen-resistant cases were included in those previous studies, while nine of ten cases in this study had not been prescribed PPA or estrogen prior. The treatment response rate in this study could not be simply compared with those reported in the previous studies. Nevertheless, this data suggests that testosterones should be substantially effective for USMI in spayed female dogs.

The actual dosage of methyltestosterone was not described in previous reports of castrated male dogs with USMI [1]. According to Plumb's veterinary drug handbook, it can be treated at 0.5 mg/kg BW p.o. *sid.* for urinary incontinence in castrated male dogs as anecdotal dosage [20]. It has also been suggested that methyltestosterone can be administered at 1.0–1.1 mg/kg BW p.o. *sid.* or *eod.* for other disorders such as hormone-responsive alopecia [20]. In this study, the drug was administered to spayed female dogs at various doses and frequencies such as 0.32–1.27 mg/kg BW p.o. *sid.* to twice a week. There was no clear correlation or tendency between methyltestosterone dosage and effectiveness, intensity, or response latency. Although the minimum effective dose was 0.32 mg/kg BW, one case treated at a dose of 0.9 mg/kg BW had a poor response. Desirable frequency is still controversial based on our results. However, several cases recovered well at *eod.* or twice a week treatment, the reduction of treatment frequency from *sid.* to *eod.* caused recurrence and resumption of *sid.* treatment improved the outcome in one patient. Taken together, no conclusions can be drawn about the optimal dosing or number of treatments necessary to achieve the best response. Presumably, the best performance should be achieved with approximately 0.5 mg/kg BW p.o. *sid.* for spayed female dogs with USMI.

Testosterone ester is also commonly used as a long-lasting prodrug of testosterone. The optimal dosage for USMI in castrated dogs is reported to be intramuscular injection at 1.0–2.2. mg/kg every 3–6 weeks as testosterone cypionate [19]. In this study, testosterone enanthate, a testosterone ester, was used instead of methyltestosterone in one case with recurrence of USMI. This case had been initially treated with methyltestosterone and showed a good response. After reduction of the treatment frequency from *sid.* to *eod.*, the symptoms recurred. Since methyltestosterone production was stopped by the manufacturer at that time, testosterone enanthate was used. Testosterone enanthate completely cleared USMI and did not have adverse effects in that case. Although this is just one case report, it is possible that testosterone enanthate could be an alternative of hormone therapy for USMI in spayed female dogs.

It is well known that testosterone has several adverse effects. According to the manufacturer's interview form of Enarmon tablet 25 mg, this drug should be contraindicated in patients with hepatic impairment or androgen-dependent carcinoma. It is also said to be used with caution in older adults. Although no published data has documented adverse effects associated with testosterone supplementation in dogs with USMI, various pathologies including perianal adenomas, perineal hernias, and prostatic hyperplasia have been associated with increased serum testosterone levels in dogs [3, 8, 15, 25]. Additionally, impairment of liver function and behavioral changes, such as excessive aggressiveness and leg raising during urination, are also predictable. However, no side effects due to testosterone administration have been found in previous reports [1, 19]. In this study, there were no clinical signs, including general behavior or activity, observed in any dog by the owners, which is consistent with those in the previous reports. However, ALT levels were mildly increased to around 100 to 300 U/l in two of the 10 dogs, several weeks after the start of treatment. One of them was 16 years old. These two dogs were treated with 1.0 and 1.1 mg/kg BW p.o. *sid.*, respectively, which were higher dosages compared with the those in other cases. In one case, the suspension of treatment for 4 weeks improved the ALT value to the reference range. Taken together, this therapy should be used with caution in dogs with liver disease and/or older dogs.

The mechanism by which testosterone is effective against USMI is still unknown. In male rats and female rabbits, androgen receptors are expressed in the urethral and bladder epithelium urethral smooth muscle [21, 22]. Rosenzweig *et al.* also suggested the possibility that androgen therapy can alleviate lower urinary tract symptoms [21]. Indeed, contractions of bladder and urethra and micturition dysfunction in elder ovariectomized female rats, were improved by treatment with testosterone [6]. In the present study, measurement of the thickness of the urethral smooth muscle in Case J was attempted using ultrasonography on a trial basis (Fig. 1). The width of the proximal urethra was 2.7 mm when the dog received a diagnosis of USMI. Interestingly, the width was enlarged to 3.1 mm 3 weeks after, and 5.0 mm 3 months after the start of administration. Although there are no reports about the





normal range of urethral thickness, and no evidence concerning changes in urethral thickness, this finding leads to the hypothesis that testosterone could up-regulate the growth and/or functions of the urethral smooth muscle (Fig. 1).

This study has several limitations. All data was retrospectively collected from medical reports and interviews with owners. The effectiveness of the drugs on USMI was evaluated by the owners, and a placebo treatment group or another drug-treatment group was not designed. Several investigators have noted a placebo effect in owners' perception of the continence of their dogs following medical intervention [7, 24]. USMI was diagnosed by exclusion of other possible causes or specific diseases in this study. Although diagnosis by exclusion is the commonly accepted method for diagnosis of USMI, the urodynamics test is also known as a definitive diagnosis method [7, 11, 18].

In summary, the efficiency and adverse effects of a single treatment of testosterone on spayed female dogs with USMI were retrospectively evaluated in this study. Nine of the ten dogs had good/excellent responses for 2 to 4 weeks. While no severe adverse effects were observed in any dog, ALT levels temporally increased in two of the ten dogs. After dose reduction or withdrawal, two of the eight dogs had recurrence of urinary incontinence. Resumption of testosterone treatment clearly improved the symptoms in those two dogs. These results indicate that testosterone reagents might be an option for treating USMI in spayed female dogs.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

ACKNOWLEDGMENT. This work was partially funded by a Grant-in-Aid for Scientific Research from JSPS (KAKENHI Grant Number 18H02340).

#### REFERENCES

- 1. Aaron, A., Eggleton, K., Power, C. and Holt, P. E. 1996. Urethral sphincter mechanism incompetence in male dogs: a retrospective analysis of 54 cases. *Vet. Rec.* **139**: 542–546. [Medline] [CrossRef]
- 2. Acierno, M. J. and Labato, M. A. 2019. Canine incontinence. Vet. Clin. North Am. Small Anim. Pract. 49: 125-140. [Medline] [CrossRef]
- 3. Applegate, R., Olin, S. and Sabatino, B. 2018. Urethral sphincter mechanism incompetence in dogs: an update. J. Am. Anim. Hosp. Assoc. 54: 22–29. [Medline] [CrossRef]
- 4. Arnold, S., Jäger, P., DiBartola, S. P., Lott-Stolz, G., Hauser, B., Hubler, M., Casal, M., Fairburn, A. and Rüsch, P. 1989. Treatment of urinary incontinence in dogs by endoscopic injection of Teflon. J. Am. Vet. Med. Assoc. 195: 1369–1374. [Medline]
- Barsanti, J. A., Edwards, P. D. and Losonsky, J. 1981. Testosterone responsive urinary incontinence in a castrated male dog. J. Am. Anim. Hosp. Assoc. 17: 117–119.
- Bonilla-Becerra, S. M., de Oliveira, M. G., Calmasini, F. B., Rojas-Moscoso, J. A., Zanesco, A. and Antunes, E. 2017. Micturition dysfunction in four-month old ovariectomized rats: Effects of testosterone replacement. *Life Sci.* 179: 120–129. [Medline] [CrossRef]
- Byron, J. K., March, P. A., Chew, D. J. and DiBartola, S. P. 2007. Effect of phenylpropanolamine and pseudoephedrine on the urethral pressure profile and continence scores of incontinent female dogs. *J. Vet. Intern. Med.* 21: 47–53. [Medline] [CrossRef]
- 8. Dow, S. W., Olson, P. N., Rosychuk, R. A. and Withrow, S. J. 1988. Perianal adenomas and hypertestosteronemia in a spayed bitch with pituitarydependent hyperadrenocorticism. *J. Am. Vet. Med. Assoc.* **192**: 1439–1441. [Medline]
- 9. Ettinger, S. J. and Feldman, E. C. 2010. Textbook of veterinary internal medicine: disease of the dog and cat. 7th ed., Elsevier Saunders, St. Louis.
- Forsee, K. M., Davis, G. J., Mouat, E. E., Salmeri, K. R. and Bastian, R. P. 2013. Evaluation of the prevalence of urinary incontinence in spayed female dogs: 566 cases (2003–2008). J. Am. Vet. Med. Assoc. 242: 959–962. [Medline] [CrossRef]
- Goldstein, R. E. and Westropp, J. L. 2005. Urodynamic testing in the diagnosis of small animal micturition disorders. *Clin. Tech. Small Anim. Pract.* 20: 65–72. [Medline] [CrossRef]
- 12. Holt, P. E. 1985. Importance of urethral length, bladder neck position and vestibulovaginal stenosis in sphincter mechanism incompetence in the

incontinent bitch. Res. Vet. Sci. 39: 364-372. [Medline] [CrossRef]

- Luster, M. I., Boorman, G. A., Korach, K. S., Dieter, M. P. and Hong, L. 1984. Mechanisms of estrogen-induced myelotoxicity: evidence of thymic regulation. Int. J. Immunopharmacol. 6: 287–297. [Medline] [CrossRef]
- 14. Mandigers, R. J. and Nell, T. 2001. Treatment of bitches with acquired urinary incontinence with oestriol. Vet. Rec. 149: 764–767. [Medline] [CrossRef]
- 15. Mann, F. A., Boothe, H. W., Amoss, M. S., Tangner, C. H., Puglisi, T. A. and Hobson, H. P. 1989. Serum testosterone and estradiol 17-beta concentrations in 15 dogs with perineal hernia. J. Am. Vet. Med. Assoc. 194: 1578–1580. [Medline]
- Nickel, R. F., Vink-Noteboom, M. and van den Brom, W. E. 1999. Clinical and radiographic findings compared with urodynamic findings in
- neutered female dogs with refractory urinary incontinence. *Vet. Rec.* **145**: 11–15. [Medline] [CrossRef] 17. Noël, S., Claeys, S. and Hamaide, A. 2010. Acquired urinary incontinence in the bitch: update and perspectives from human medicine. Part 2: The urethral component, pathophysiology and medical treatment. *Vet. J.* **186**: 18–24. [Medline] [CrossRef]
- Owen, L. J. 2019. Ureteral ectopia and urethral sphincter mechanism incompetence: an update on diagnosis and management options. J. Small Anim. Pract. 60: 3–17. [Medline] [CrossRef]
- 19. Palerme, J. S., Mazepa, A., Hutchins, R. G., Ziglioli, V. and Vaden, S. L. 2017. Clinical response and side effects associated with testosterone cypionate for urinary incontinence in male dogs. *J. Am. Anim. Hosp. Assoc.* **53**: 285–290. [Medline] [CrossRef]
- 20. Plumb, D. C. 2015. Plumb's Veterinary Drug Handbook, 8th ed., PharmaVet, Stockholm.
- 21. Rosenzweig, B. A., Bolina, P. S., Birch, L., Moran, C., Marcovici, I. and Prins, G. S. 1995. Location and concentration of estrogen, progesterone, and androgen receptors in the bladder and urethra of the rabbit. *Neurourol. Urodyn.* 14: 87–96. [Medline] [CrossRef]
- 22. Salmi, S., Santti, R., Gustafsson, J. A. and Mäkelä, S. 2001. Co-localization of androgen receptor with estrogen receptor beta in the lower urinary tract of the male rat. J. Urol. 166: 674–677. [Medline] [CrossRef]
- 23. Salomon, J. F., Cotard, J. P. and Viguier, E. 2002. Management of urethral sphincter mechanism incompetence in a male dog with laparoscopicguided deferentopexy. J. Small Anim. Pract. 43: 501–505. [Medline] [CrossRef]
- 24. Scott, L., Leddy, M., Bernay, F. and Davot, J. L. 2002. Evaluation of phenylpropanolamine in the treatment of urethral sphincter mechanism incompetence in the bitch. J. Small Anim. Pract. 43: 493–496. [Medline] [CrossRef]
- Sirinarumitr, K., Johnston, S. D., Kustritz, M. V., Johnston, G. R., Sarkar, D. K. and Memon, M. A. 2001. Effects of finasteride on size of the prostate gland and semen quality in dogs with benign prostatic hypertrophy. J. Am. Vet. Med. Assoc. 218: 1275–1280. [Medline] [CrossRef]