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TESTOSTERONE HAD NEGATIVE IMPACT ON VOIDING FUNCTION OF THE CONSCIOUS MICE LACKING M3 MUSCARINIC ACETYLCHOLINE RECEPTOR: CHRONOLOGICAL EVALUATION OF POST-VOID RESIDUAL EVALUATED BY MINIATURE HIGH-FREQUENCY TRANSRECTAL ULTRASOUND

Hypothesis / aims of study
We developed the novel in vivo imaging analysis of the urinary function to monitor postvoid residual (PVR) in conscious mice using miniature ultrasound probe with transrectal approach (1). This minimally invasive repeatable technique in measurement of PVR after voluntary void of the conscious mice revealed excellent accuracy to identify and monitor the voiding function. Aims of this study is to evaluate the possible effects of gender and testosterone on voiding function in the mice lacking M3 muscarinic acetylcholine receptors (M3KO mice).

Study design, materials and methods
The M3KO mice (n=82 in total), generated at least 10 generations and age-matched C57BL/6 wild-type mice (WT mice, n=32) (2), were used in the experiments. Voided volume was measured by weight gain of paper with which urine was wiped just after voiding. PVR was measured by using 2-mm in diameter 20MHz ultrasound probe transrectally (ALOKA, Japan). Total bladder volume was calculated by sum of voided volume and PVR. We measured voided volumes and PVRs weekly in the 4 experiments as follows. In the experiment [1] to test possible difference of voiding function by gender according to the age, both WT (number of male/female = 16/16) and M3KO mice (number of male/female = 15/15) were evaluated from 5 to 50 weeks of age and compared between male and female. In the experiment [2] to test voiding function in castrated male mice, 10 weeks old male M3KO mice (n=10) were castrated and compared with sham operation (n=10). In the experiment [3] to test effect of testosterone in castrated male mice, testosterone was administered to castrated male M3KO mice (n=8) after 4 weeks of castration and compared with the mice without testosterone treatment (n=8). In the experiment [4] to test effect of testosterone in female mice, testosterone was administrated to 10 weeks old female M3KO mice (n=8) and compared with the mice without testosterone treatment (n=8).

Results
Experiment [1]: Male WT mice had significantly greater PVR than female WT mice in 5-50 weeks (ANOVA $p<0.001$, Fig.1). There was no significant difference between male and female WT mice in voided volume and total bladder volume (ANOVA $p=0.87$, 0.31, respectively). Male M3KO mice had significantly greater PVR, voided volume, and total bladder volume than female M3KO mice in 5-50 weeks (ANOVA $p<0.001$ for all, Fig.2). Importantly, PVR in male M3KO mice increased with aging (correlation co-efficiency of $r=0.9637$, $p<0.001$).
Experiment [2]: Castration caused significant decrease of PVR in male M3KO mice comparing with sham operated mice from 4 weeks after treatment (ANOVA $p<0.001$, Fig.3).
Experiment [3]: Administration of testosterone to castrated male M3KO mice increased their PVRs (ANOVA $p<0.001$, Fig.4).
Experiment [4]: Administration of testosterone to female M3KO mice did not alter their voided volumes, PVRs nor total bladder volumes.

Interpretation of results
Increase of PVR in male mice was remarkable in both WT and M3KO mice. The difference of PVR in male mice was more prominent in M3KO than WT mice. Importantly, PVR in male M3KO increased with aging; on the other hand, female M3KO mice had no increase of PVR during entire age. Interestingly, castration caused significant decrease of PVR in male M3KO mice, suggesting that the voiding dysfunction of male M3KO mice was controlled through certain hormonal mechanisms. Our study further revealed that administration of testosterone in castrated M3KO male increased PVR and the effect of testosterone was reversible. On the other hand, there was no effect of testosterone on PVR in female M3KO mice; which suggested that testosterone might inhibit the compensatory mechanism of the voiding dysfunction in male M3KO mice through binding androgen receptor.

Concluding message
We firstly demonstrated gender difference of voiding function in both WT and M3KO mice, represented by increase of PVR in male mice by chronological monitoring using transrectal ultrasonography. Lack of M3 receptor had significant negative impact on voiding function in male mice by represented the increase of PVR; however, no impact on PVR in female, which suggest that there may exist certain compensatory mechanism of voiding function in M3KO female mice. Castration in M3 KO male mice had positive impact on decrease of the pathological PVR, and testosterone was significantly involved in the voiding dysfunction in male M3KO mice. The compensatory mechanism of M3KO female mice in the voiding function needs to be clarified in further study.

Fig.1

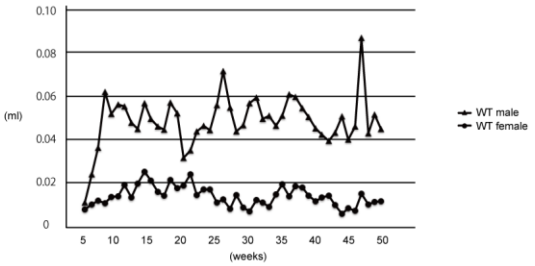


Fig.2

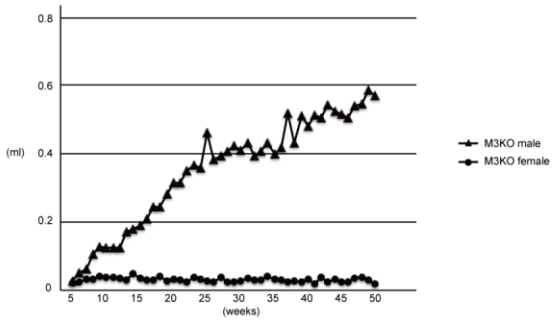


Fig.3

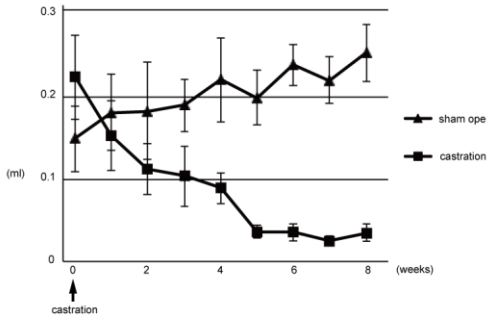
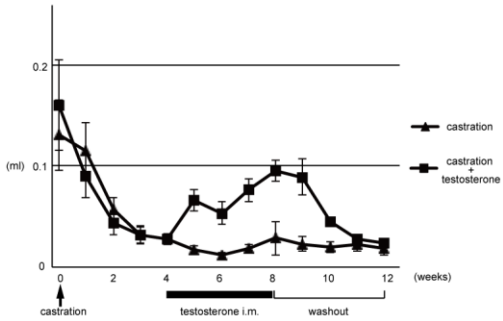


Fig.4



References

1. ICS 2007 Abstract # 200
2. Proc Natl Acad Sci U S A, 97: 9579, 2000

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**The animal ethics committee of Kyoto Prefectural University of Medicine**