NOVEL QUANTIFICATION METHOD USING VOIDED STAIN ON PAPER TECHNIQUE FOR VOIDING ANALYSIS IN THE MOUSE

Hypothesis / aims of study
In the investigation of voiding dysfunction, the mouse has not been a popular model animal for the difficulty in quantifying minute voided urine. We developed a simple and reliable quantification method; Voided Stain on Paper (VSOP) method. The utility of the method were evaluated in mice with cyclophosphamide (CPM)-induced cystitis. Further, age-dependent changes in voided volume were analyzed in mice aged between two to thirteen weeks.

Study design, materials and methods
Voided Stain on Paper (VSOP) method: The amount of voided urine was assessed as liquid stain area on a filter paper (FILTER PAPER QUALITATIVE ADVANTEC 240mm: Toyo Roshi Kaisha, Ltd., Japan). Voiding behavior of mice was analyzed by placing the animal above the filter paper and recording voided time and area over two hours. Before each analysis, mice were fed with 50μl/g distilled water to maintain the same level of hydration among animals. The recorded stain areas were calculated by computer software Photoshop (Adobe, USA).

(1) Generation of a standard correlation formula. The correlation between predefined amount of saline and stained area was calculated, and used as a standard formula for further mouse studies.
(2) CPM cystitis model: Five male and 5 female seven-week-old ddY mice (Clea Japan, Inc., Japan) were treated with CPM (150mg/kg) intraperitoneally. As control groups, the same volume of saline was injected intraperitoneally to 5 male and 5 female mice of the same age. Twenty hours later, voiding behavior was analyzed by the VSOP method. Thereafter, sections of the bladders were evaluated histologically to confirm CPM-induced cystitis, which was diagnosed by the presence of severe submucosal edema.
(3) Age-dependent voiding development: Voiding behavior of 2, 4, 7, 10 and 13-week old male and female ddY mice (six to ten/each group) was recorded using the VSOP method.

Results
(1) There was a linear correlation between liquid volume and stained area on the filter paper within the range of 50 to 800 μl (y=16.472x-22.411, R²=0.9981).
(2) Voiding behavior of CPM cystitis model: No control mice showed cystitis histologically. In CPM-treated male mice, histological cystitis was noted in 4/5, and the voiding volume was significantly lower than controls (195.6±36.3 and 413.9±28.0 l for CPM and control, respectively (p=0.0014) ). In CPM-treated female mice, cystitis was histologically proven only in 3/5, with a marginal difference in voided volume between two groups (197.7±79.9 and 362.7±23.2 l for CPM and control, respectively (p=0.082) ). These findings indicate the consistent correlation between histological cystitis and reduced voided volume.
(3) Voiding development of the mouse: Voided volume of ddY mice was able to be recorded as early as 2 week-old, and increased along with their growth (Fig 1).

Interpretation of results
Voided Stain on Paper (VSOP) method is reliable in recording minute urination volume in the mouse. This method could be a useful tool to analyze voiding behaviour in pathological conditions such as chronic cystitis, in which quantification of reduced voided volume is challenging. Most notably, age-dependent increases in voided volume starting from a weaning period are reported for the first time in normal mice. Therefore, this method can provide fundamental basic data for the analysis of voiding phenotypes of various pathological murine models, including genetically-modified mice.

Concluding message
The VSOP method is a novel and reliable voiding quantification technique in the mouse. This simple method will be a powerful tool for evaluating voiding phenotypes in mouse models of lower urinary tract dysfunction.

Fig 1
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ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by Animal Research Committee, Kyoto University