Title of Research:

15_PT01-01

Development of new *in vivo* screening method to validate the low-dose effects induced by estrogenic chemicals using estrogen reporter mice

Principal Investigator:

Tsuyoshi Nakanishi (Laboratory of Hygienic Chemistry and Molecular Toxicology, Gifu Pharmaceutical University)

Collaborators:

Hisamitsu Nagase (Laboratory of Hygienic Chemistry and Molecular Toxicology, Gifu Pharmaceutical University)

Summary of Research:

Background: The uterotrophic bioassay has been proposed as a short-term *in vivo* screening test to detect the estrogenic properties of potentially endocrine disrupting chemicals by the Organisation for Economic Co-operation and Development (OECD). Although the test is already standardized as a OECD test guideline (Test No.440), there is a possibility that the test might overlook a certain estrogenic properties which has no effect to the uterus. On the other hand, we recently generated a new transgenic mouse carrying “E-Rep” reporter gene which consisted of the luciferase (Luc) under the control of estrogen-responsive elements coupled to a minimal promoter. E-Rep mouse is a potential powerful tool for assessing the kinetics of gene activation by estrogenic properties *in vivo*. In the 4th term, we have established a provisional protocol for the novel *in vivo* screening test, which employs bioluminescence imaging in the whole body as the end point for estrogenic action of test chemicals.

Objective: In the current term, we verified the validity of the provisional protocol by confirming whether the estrogen-induced bioluminescence imaging in the whole body reflects the estrogenic action in each organ. In addition, using the bone tissue-specific selective estrogen receptor modulators (SERM), which have little effect on the uterus, we confirmed whether our novel *in vivo* screening test have an advantage over the uterotrophic bioassay.

Methods: The experimental protocols of the new assay using E-Rep mice are based on the uterotrophic bioassay with some modifications. Briefly, E-Rep female mice (8 weeks of age) were ovariectomized and then fed phytoestrogen-free purified diet during the experimental period. After 7 days of ovariectomy, the mice were orally gaveged with 17α-ethinylestradiol (EE) or SERMs for 7 consecutive days. The Luc activity was monitored in living animals with IVIS imaging at Day 1, 2, 4 and 7. After last detection of *in vivo* bioluminescence imaging, mice were dissected and measured uterine weight in accordance with the uterotrophic bioassay. For measurement of daily Luc activity induced by EE in each organ, EE-gaveged mice were dissected and removed each organ at Day 1, 2, 4 and 7.

Results: According to measurement of daily Luc activity in each organ, it was confirmed that the EE-induced bioluminescence imaging in the whole body reflects its estrogenic action in each organ. Furthermore In the experiment using the bone tissue-specific SERMs, our novel *in vivo* screening test could determine the SERMs as positive estrogens, whereas the uterotrophic bioassay could not determine a SERM even at a dose which show sufficient estrogen activity in the bone tissue.

Conclusion: Our novel *in vivo* screening test using E-Rep mice with *in vivo* bioluminescence imaging is an excellent method that can detect SERMs that cannot be detected by the uterotrophic bioassay.

Timeline: April, 2017 – March, 2018

Topics:

1) Dr. Tsuyoshi Nakanishi was awarded the 4th JCIA-LRI award for the research theme "Comprehensive research on reproductive toxicity of nuclear receptor-activated hazards and establishment of its evaluation system".

Publications:

1) Nishioka Y, Tamai K, Onda M, Hiromori Y, Kimura T, Hu J, Nagase H, Nakanishi T, Potential interference of oil vehicles on genital tubercle development during the fetal period in ICR mice, ***Biol. Pharm. Bull.***, 41:266-271 (2018)