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Effects of various drugs on bilateral oophorectomy -induced climacteric disorder model in rats

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Summary in Japanese

閉経後の女性には卵巣機能の低下によるエストロゲンの減少に伴って更年期障害と呼ばれる様々な不定愁訴が現れる．その代表的な症状として顔や胸のほてり，更年期うつ，骨粗鬆症などがある．本研究では，雌性 SD ラットの両側の卵巣を摘出することで更年期障害様モデルを作製した．モデルラットでは偽処置群と比較して摂餌量，体重および尾部表面温度が有意に上昇し，脛骨における海綿骨の減少や強制水泳試験における無動時間の延長も認められた．
本モデルに対し，ホルモン補充療法を目的とした 17β- エストラジオール（E2），血管運動神経症状に効果が期待されるパロキセチンおよび大豆イソフラボンの一種であるゲニステインを投与した結果，更年期障害様の症状は E2 投与群で抑制された．これらの結果より，本評価系は更年期障害様モデルを用いた治療薬の検討に有用であると考えられた．

Objective

Postmenopausal women exhibit a variety of nonspecific complaints due to a decrease in the estrogen levels, caused by reduced ovarian functions. This is commonly referred to climacteric disorders.

A representative symptom is hot flashes (HF) as in the face and chest. In addition to body temperature changes, osteoporosis, and climacteric depression are also known. In this study, we attempted to preparate a climacteric disorders-like model and to evaluate the therapeutic effects of representative drugs. Female SD rats were bilaterally ovariectomized. We observed 1) the general conditions such as food intake, body weight, tail skin temperature, 2) cancellous bone at the proximal end of the tibias of foot, and 3) depression-like syndrome using the forced swimming test. The therapeutic effects of estradiol (E2，as a hormone replacement therapy), paroxetine (for treating vasomotor symptoms), and genistein (a type of soy isoflavone) were also tested.

Materials and Methods

Animal

Crl:CD(SD), female, 10 weeks old (At the time of model preparation)

Methods of model preparation

Rats were anaesthetised with sevoflurane (2-4%) and bilateral ovaries were removed. Vaginal smears were collected for 6-7 consecutive days from the day after ovariectomy in all animals used for model preparation to confirm that the sexual cycle had ceased.

Tail skin temperature：TST

The thermometer probe connected to a digital thermometer (Unique Medical Co.,Ltd.) was wound around the base of the animal's tail at the distance of approximately 2 cm to measure the temperature every minute on before ovariectomy and 1,2,3,4W after ovariectomy (individual values: average value over 30 min)

Forced swimming test：FST

At 5 W after ovariectomy, the rats were placed in a water tank and forced to swim for 15 min (measurement acclimatization). 24 hours later of measurement acclimatization, the rats were placed back in the water tank and forced to swim for 5 min, during which the immobility time was assessed as depressive-like behavior. At 9W and 13W after ovariectomy, rats were forced to swim for 5 min without performed measurement acclimatization and the 5-min immobility time was assessed.

Bone analysis using μCT

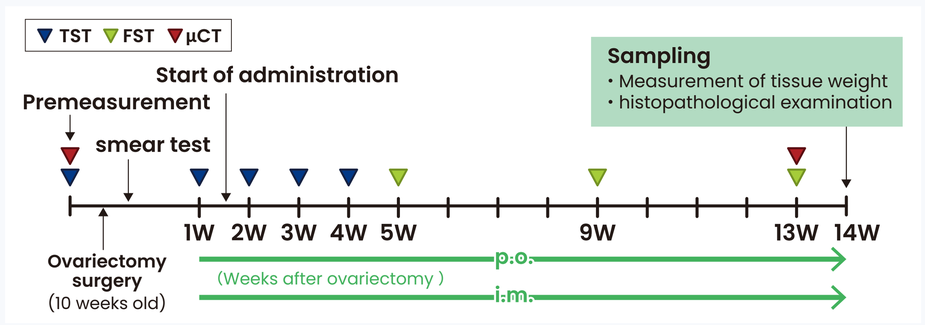
CT images of the upper tibia of the right foot were taken before ovariectomy and 13W after ovariectomy under sevoflurane (2-4%) anaesthesia using CosmoScan GX II (Rigaku Corporation). Bone Analysis (Rigaku Corporation) was used for CT image analysis.

Micro-X-ray CT system
CosmoScan GX II

Tissue weights and histopathological examination

The uterus, peri-uterine white fat and right tibia were collected at 14 W after ovariectomy. The uterus and peri-uterine white fat were weighed. The uterus, peri-uterine white fat and right tibia were examined histopathologically by haematoxylin and eosin staining.

Study schedule



Test Group	Dose (mg/kg)	Route of Administration	Dosing frequency	Number of animals
Sham※	0	p.o.	Once a day	6
Vehicle control※	0	p.o.	Once a day	6
E2 low dose	0.1	i.m.	Once a week	6
E2 high dose	0.3	i.m.	Once a week	6
Paroxetine	10	p.o.	Once a day	6
Genistein	50	p.o.	Once a day	6

※The sham group and vehicle control group were orally administered saline.

Conclusion

- The removal of bilateral ovaries from female SD rats allowed us to capture various symptoms of menopause.
- Compared to the vehicle control group, the E2 administered groups had inhibited the increase in the TST, body weight gain, decrease in trabecular bone and decrease in uterine weight.
- In the paroxetine group, no significant differences were observed in the TST or the immobility time of the FST, but the values were low.
- In the genistein group, prolonged immobility time in FST and reduction in uterine weight were inhibited. In addition, the TST was lower in the genistein group, although there were no significant difference.

These results suggest that this evaluation system is useful for investigating therapeutic agents using the climacteric disorder-like model.

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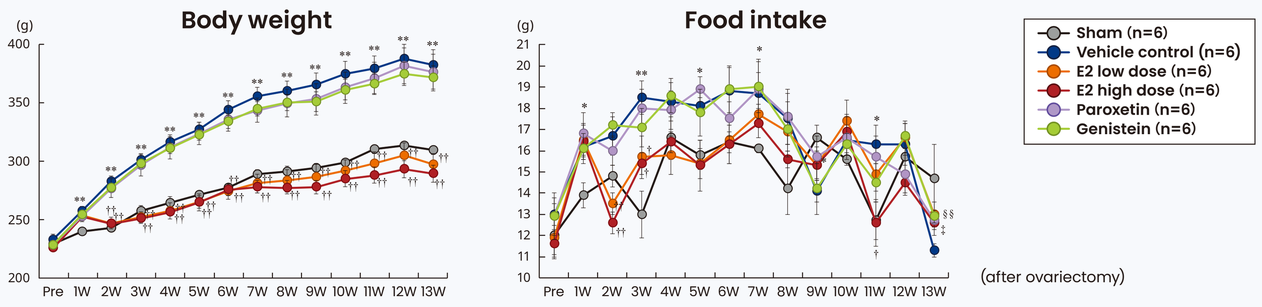
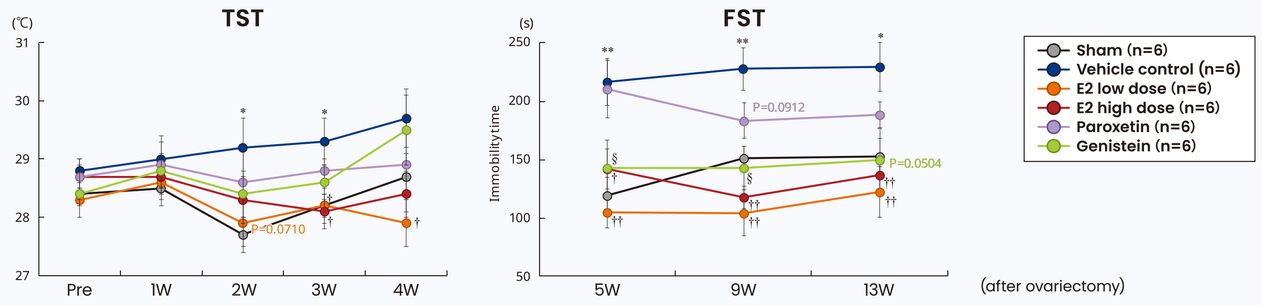
Results

Body weight and food intake



Mean ± S.E.M.
*: p<0.05, **: p<0.01 Sham group vs Vehicle control group (Student's *t*-test)
†: p<0.05, ††: p<0.01 Vehicle control group vs E2 low dose group , E2 high dose group (Dunnett's multiple comparison test)
‡: p<0.05 Vehicle control group vs Paroxetine group (Student's *t*-test)
§§: p<0.01 Vehicle control group vs Genistein group (Student's *t*-test)

Tail skin temperature (TST) and Forced swimming test (FST)



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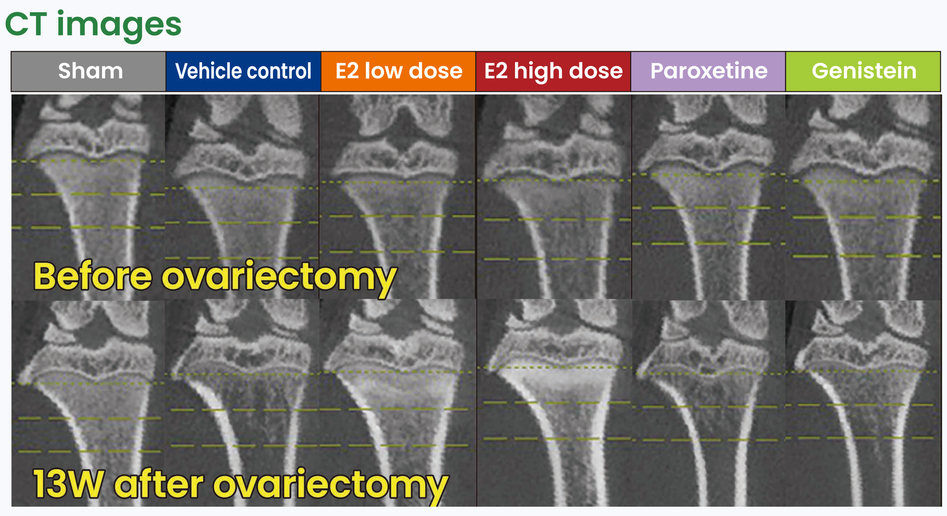
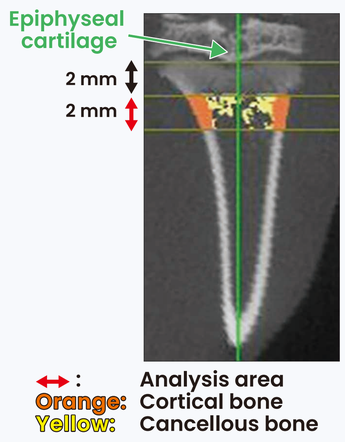
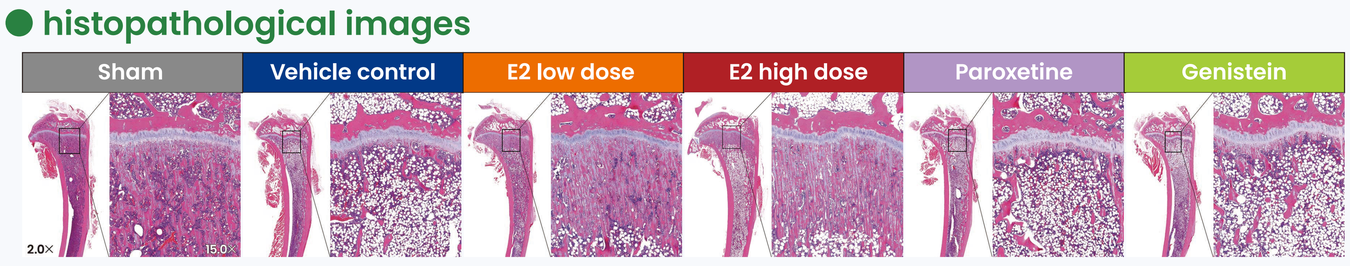
Bone analysis using μCT

CT images

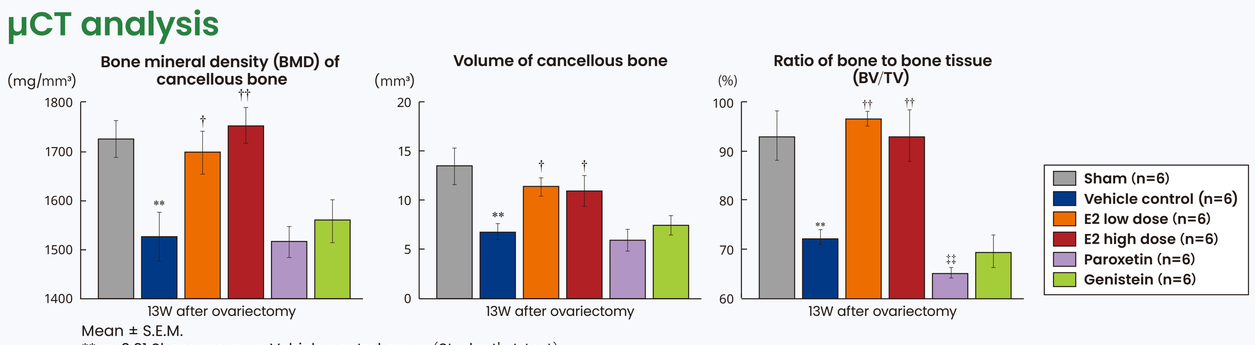




histopathological images



μCT analysis



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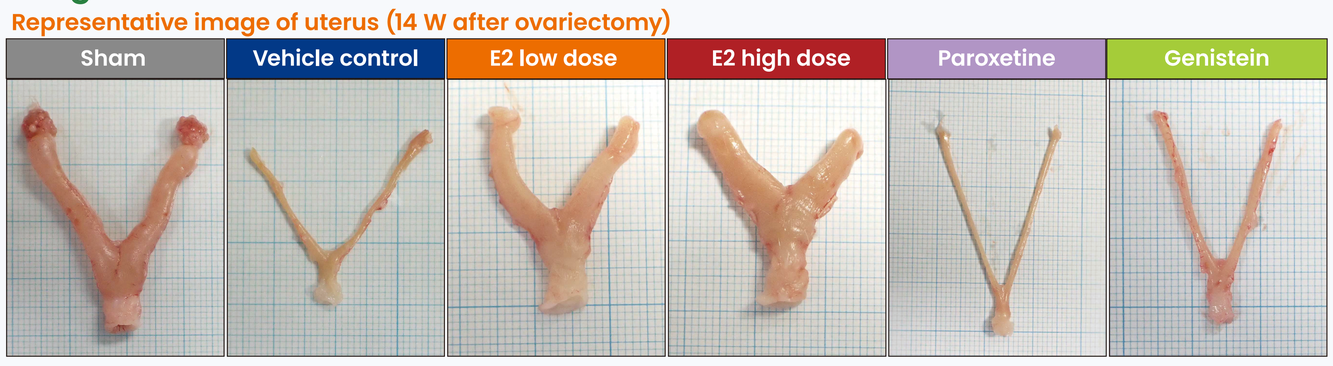
No significant difference Vehicle control group vs Genistein group (Student's *t*-test)

Evaluation of tissue weights

Drug evaluation for the uterus

Representative image of uterus (14 W after ovariectomy)



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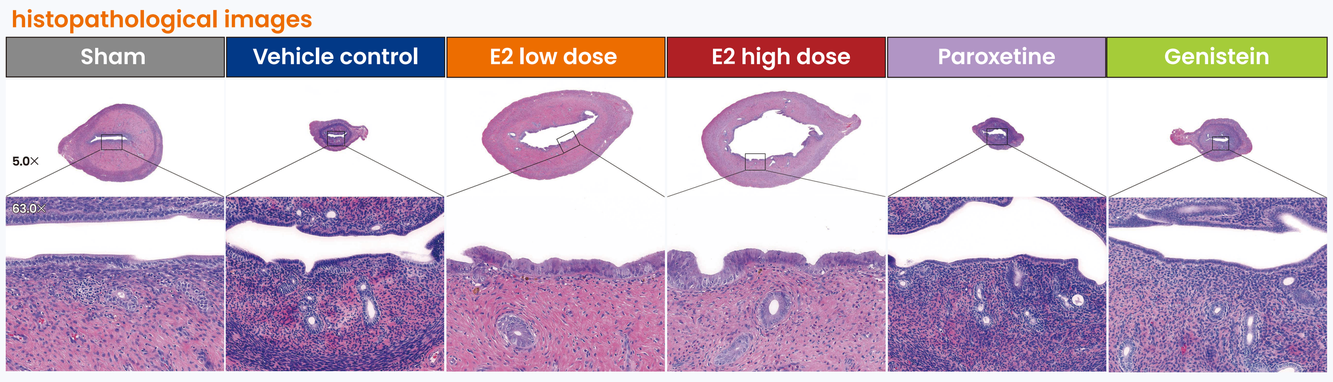

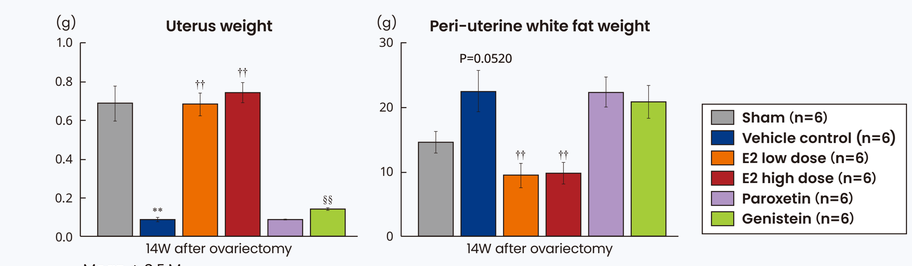

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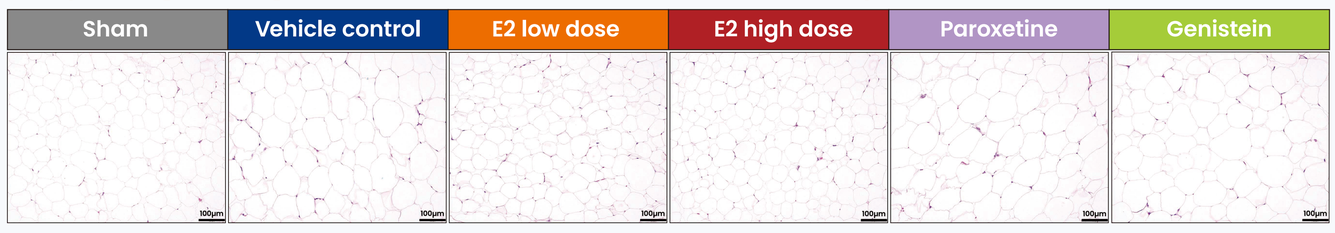
Drug evaluation for Peri-uterine white fat

histopathological images of Peri-uterine white fat (14 W after ovariectomy)



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