

Dietary Phytoestrogen Reduces Intracranial Aneurysm Formation in Ovariectomized Female Mice

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Introduction

- 1. Postmenopausal women have a higher incidence of intracranial aneurysmal rupture than men of the same age.
- 2. Previous studies showed that estrogen can protect against intracranial aneurysm formation and rupture through the activation of estrogen receptor-ß (ER-ß) (Hypertension 63:1339-1344, 2014).
- 3. Equal, a phytoestrogen produced by the gut microbiota from dietary daidzein, is known to preferentially bind to ER-B.

Methods

Mice: 8 weeks old, C57BL/6J female 1. Equal study: Vehicle Control (VC, n=30), equol 0.5mg/day s.c. (Equol, n = 30)

2. Daidzein study 1: Isoflavone free diet group (non-Iso, n=30), 0.1% daidzein diet group (Daidzein, n=30) 3. Daidzein study 2: Daidzein + Vancomycin (Daidzein + VCM, n=20),

Daidzein + VCM + Equol (n=20)

Aim of the study

- 1. To verify equal supplement reduces intracranial aneurysm formation in ovariectomized female mice.
- 2. To verify dietary daidzein reduces intracranial aneurysm formation in ovariectomized female mice via gut microbiota.

Protocol

- 1. Bilateral ovariectomy
- 2. Aneurysmal induction:
 - Unilateral nephrectomy
 - Deoxycorticosterone acetate salt (DOCA-salt) hypertension, DOCA 66mg/28days
 - Elastase injection (35mU) into the cerebrospinal fluid at right basal cistern

3. **Diet**:

- Isoflavone-free diet (AIN-93G)
- Isoflavone-free with 0.1% daidzein diet

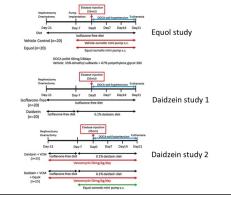
4. Neurological examination

Mice were assessed neurological symptoms once a day after aneurysm induction.

5. Criteria of euthanasia

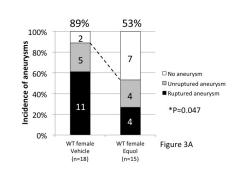
Mice were euthanized when they developed neurological symptoms. All asymptomatic mice were euthanized 21 days after aneurysmal induction.

Figure 1. Protocol



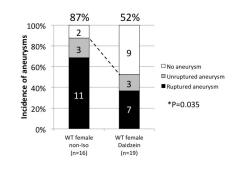
Results

Figure 2A. Incidence of aneurysms: **Equol study**



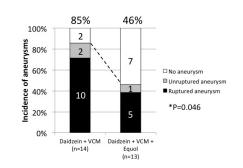
Equol significantly reduced intracranial aneurysm formation.

Figure 2B. Incidence of aneurysms: Daidzein study1



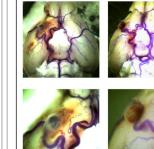
Daidzein significantly reduced intracranial aneurysm formation.

Figure 2C. Incidence of aneurysms: Daidzein study2



Daidzein with VCM had high incidence of aneurysms, but adding on equol significantly reduced intracranial aneurysm formation.

Figure 3. Representative aneurysms



Conclusion

- 1. Dietary phytoestrogen significantly reduced intracranial aneurysm formation in ovariectomized female mice.
- 2. Inhibiting gut microbiota by vancomycin increased intracranial aneurysm formation.