

**Doxycycline ameliorates aortic lesions in a mouse model for the vascular type of Ehlers-Danlos syndrome**

Wilfried Briest, Timothy K. Cooper, Hyun-Jin Tae, Melissa Krawczyk, Nazli B.

McDonnell, Mark I. Talan

The Journal of Pharmacology and Experimental Therapeutics

**Supplementary Methods:**

**Quantification of collagen content in skin**

Collagens were extracted from the skin by digestion with pepsin (Sigma-Aldrich) 1 mg/ml, in 0.5 M acetic acid and 0.2 M NaCl at RT over night. The collagen extracts were resolved with non-reducing 8% SDS/PAGE. The gel was stained with Imperial Protein Stain (Thermo Scientific, Bonn, Germany) and scanned for quantification.

**Biomechanical Properties of the Colon**

An approximately 13 mm long section of the transverse colon was removed, tied at both ends by ligatures and injected with incremented volumes of PSS by a Hamilton syringe. The luminal pressure was measured with a pressure transducer (Millar Instruments Inc., Houston, TX) and recorded by the PowerLab system (ADInstruments, Colorado Springs, CO) until the rupture pressure was reached. Biomechanical properties of colon were characterized as a maximum holding pressure (before rupture) and as compliance (pressure/volume).

## Supplemental Figure Legend

**Supplement Figure 1** Luminal radius (A) and the thickness of the tunica media (B) of the abdominal aorta in heterozygous mice (+/-) was not different from wild-type (+/+).

These values were not changed by doxycycline treatment. Data are means  $\pm$ SEM (numbers of experiment are indicated in the columns).

**Supplement Figure 2** Collagen type I content in the skin of heterozygous mice (+/-) was not different from wild-type (+/+) with or without doxycycline treatment (doxy<sub>100</sub>).

(A) Collagens were resolved by SDS/PAGE and stained with Coomassie blue. The indicated  $\beta^{1,1}$  and  $\beta^{1,2}$  dimers and  $\alpha 1(I)$  and  $\alpha 2(I)$  chains are from type I collagen.

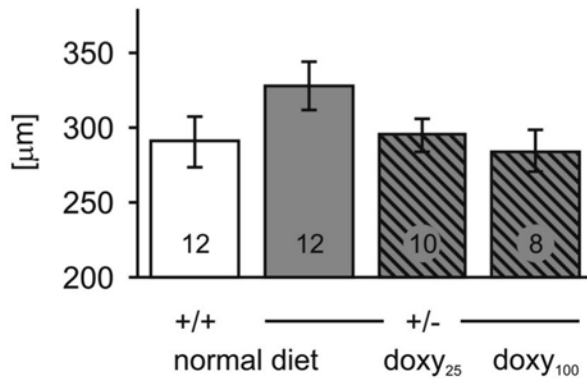
(B) Densitometric analysis revealed no differences in collagen content. Data are means  $\pm$ SEM (numbers of experiment are indicated in the columns).

**Doxycycline ameliorates aortic lesions in a mouse model for the vascular type of Ehlers-Danlos syndrome**

Wilfried Briest, Timothy K. Cooper, Hyun-Jin Tae, Melissa Krawczyk, Nazli B. McDonnell, Mark I. Talan  
The Journal of Pharmacology and Experimental Therapeutics

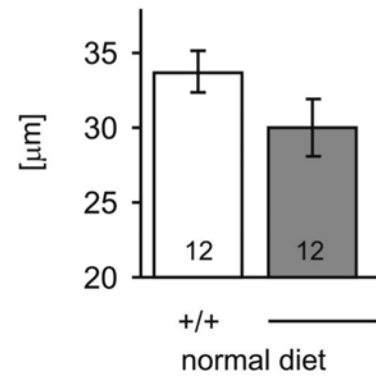
**A**

**Lumen radius**



**B**

**T. Me**

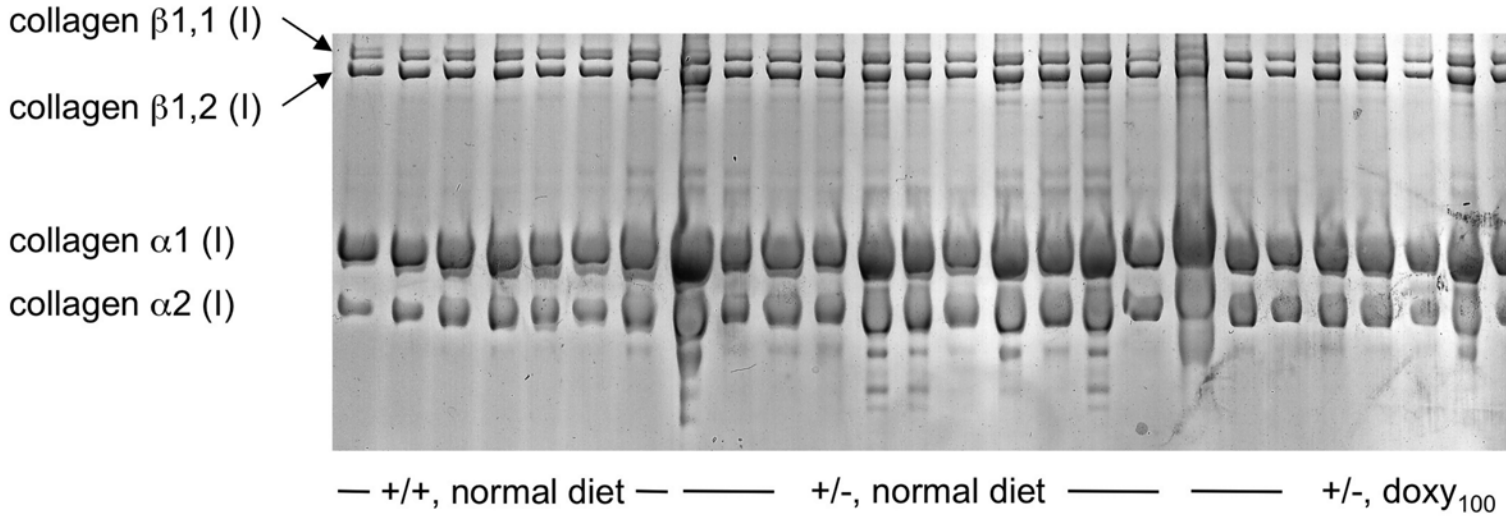


**Supplement Figure 1** Luminal radius (A) and the thickness of the tunica media (B) of the abdominal aorta in heterozygous mice (+/-) was not different from wild-type (+/+). These values were not changed by doxycycline treatment. Data are means  $\pm$ SEM (numbers of experiment are indicated in the columns).

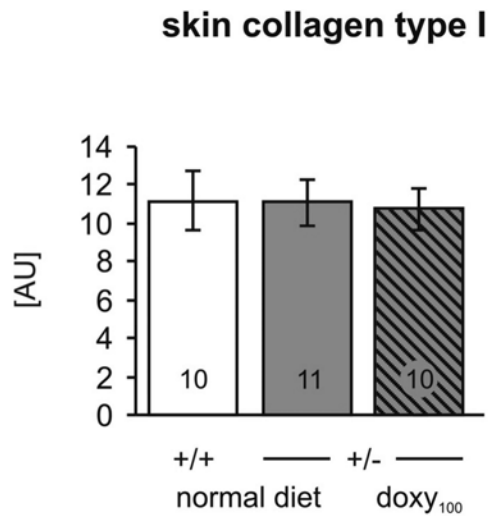
**Doxycycline ameliorates aortic lesions in a mouse model for the vascular type of Ehlers-Danlos syndrome**

Wilfried Briest, Timothy K. Cooper, Hyun-Jin Tae, Melissa Krawczyk, Nazli B. McDonnell, Mark I. Talan  
 The Journal of Pharmacology and Experimental Therapeutics

**A**



**B**



**Supplement Figure 2** Collagen type I heterozygous mice (+/-) was not different with or without doxycycline treatment. **(A)** Collagens were resolved by SDS/PAGE and stained with Coomassie blue. The indicated  $\beta 1,1$  and  $\beta 1,2$  and  $\alpha 1$  and  $\alpha 2$ (I) chains are from type I collagen. **(B)** Densitometric analysis revealed no difference in skin collagen type I content. Data are means  $\pm$ SEM (number of mice indicated in the columns).