SUPPLEMENTAL MATERIAL

Supplemental Methods

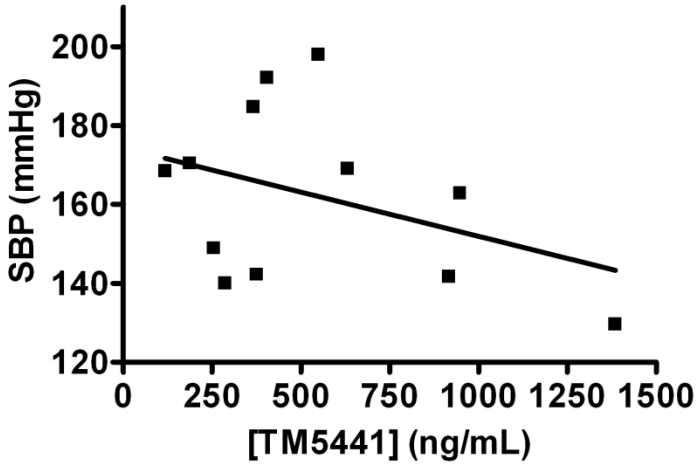
TM5441 LC/MS/MS: Samples were separated initially on a phenomenex column (C18, 1.7µm, 2.1 x 50 mm column, Waters Corporation, Milford, Massachusetts, USA) run by a Shimadzu HPLC system (Shimadzu corp., Kyoto, Japan) using a gradient of acetonitrile (ACN) with 0.1% formic acid. Mass spectrometry detection was performed on an Applied Biosystems API-4000 MS/MS system (Applied-Biosystems, Foster City, California, USA) with an atmospheric pressure electrospray ionization source. Analyst 1.5 software packages were used to control the LC-MS/MS system, acquire data, and analysis. All analyses were carried out in positive ionization with spray voltage set at 5500 V. The heated capillary temperature was set at 550 ˚C. The curtain gas, ion source gas 1, ion source gas 2, entrance potential, collision exit potential, and declustering potential were set at 10, 55, 55, 10, 10, 70 Arb, respectively. The collision energy was set at 20 and 25 for TM5441 and IS, respectively. For quantification, multiple reactions monitoring (MRM) was utilized for the transitions 429.1 *m/z* → 230.5 *m/z* for TM5441 and 429.1 *m/z* → 230.5 *m/z* for IS.

A stock solution of TM5441, 1 mg/mL, was prepared in DMSO. TM5441 standard solutions that ranged from 50 ng/mL to 250,000 ng/mL were prepared by serial dilutions in DMSO. The indomethacin (IS) stock solution was prepared in ACN with a final concentration of 1 mg/mL. The calibration curve was prepared by spiking 10 µL of the appropriate intermediate analytical standard into 490 µL blank mouse plasma to yield a concentration range of 1 ng/mL to 5,000 ng/mL. The quality control (QC) samples were prepared similarly at concentrations of 10 ng/mL and 1,000 ng/mL in mouse plasma by separately weighed compound.

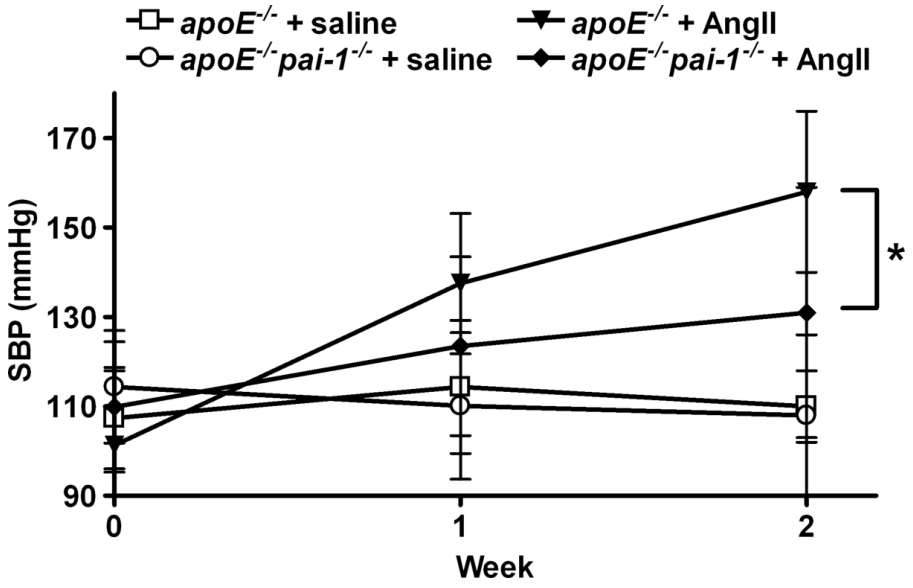
A 50 µL aliquot of plasma was transferred to a 2 mL eppendorf micro centrifuge tube and 200 µL of cold IS solution was added. For double blank samples, a 50 µL aliquot of blank plasma was added to 200 µL of cold ACN. The samples were then vortexed for 10 mins, followed by centrifugation at 14k rpms and 4°C for 10 mins and 100 µL of supernatant was collected in a 96-well plate. A 2µL aliquot was injected into the LC-MS/MS system for analysis. The QC samples were injected after every six unknown samples.

Angiotensin II Blood Pressure: apoE$^{-/-}$ and pai-1$^{-/-}$ mice, both of which are in the C57BL/6J background, were purchased from Jackson Laboratories (Bar Harbor, ME) and then crossed to generate apoE$^{-/-}$pai-1$^{-/-}$. Saline and angiotensin II (AngII) solutions were infused into either apoE$^{-/-}$ or apoE$^{-/-}$pai-1$^{-/-}$ using osmotic minipumps (Alzet, type 1002, Durect Corporation, Cupertino, CA) that were implanted subcutaneously. Minipumps were loaded with Ang II resuspended in 0.9% sterile saline solution to deliver a dose of 600 ng/kg/min for 14 days. Mice in the control group received saline only. Systolic blood pressure was measured in conscious mice once a week using a non-invasive tail-cuff system (BP 2000, Visitech Systems, NC). Animals were habituated to the measurement conditions (under a restrainer on temperature controlled platform for up to one hour) for 3 days before recording the baseline measurements. Three sets of 10 measurements were recorded for each animal approximately at the same time of the day.

Supplemental Figures and Legends



Supplemental Figure 1. SBP as a function of plasma levels of TM5441. LC/MS/MS measurements were used to confirm the presence of TM5441 in the plasma from L-NAME-treated animals. The average concentration in the WT + L-NAME + TM5441 group was 535 ng/mL. The amount of TM5441 correlated with the reduction of SBP, though not significantly. R^2= 0.1448.



Supplemental Figure 2. PAI-1 deficiency protects against angiotensin II-induced hypertension. Mice were administered either angiontensin II (AngII) or saline via osmotic mini-pump for 2 weeks. PAI-1 deficiency attenuated the increase in SBP due to AngII. ApoE deficiency has no effect on SBP and should be regarded as identical to WT animals. Data are mean ± SD. n=12. *P=0.01.