

Supplementary Information

**RTA 405, A SYNTHETIC TRITERPENOID, INCREASES GLOMERULAR
FILTRATION RATE AND REDUCES ANGIOTENSIN II-INDUCED CONTRACTION
OF GLOMERULAR MESANGIAL CELLS**

Running Title: RTA405 modulates GFR by inhibiting MC contraction

**Yanfeng Ding¹, Rhesa Stidham², Ron Bumeister², Isaac Trevino², Ali Winters¹, Marc
Sprouse¹, Min Ding¹, Deborah A. Ferguson², Colin J. Meyer², W. Christian Wigley² and
Rong Ma^{1*}**

1: Department of Integrative Physiology and Cardiovascular Research Institute

University of North Texas Health Science Center, Fort Worth, TX

2: Reata Pharmaceuticals Inc., Irving, TX

*: To whom correspondence should be addressed:

RES-302G, 3500 Camp Bowie Blvd.

Department of Integrative Physiology

University of North Texas Health Science Center

Fort Worth, TX 76107

Tel: 817-735-2516; Fax: 817-735-5084

E-mail: rong.ma@unthsc.edu

FIGURE LEGENDS

Fig. S1. RTA 405 induced expression of Nrf2 target genes in cultured rat mesangial cells with and without Ang II treatment.

Rat Primary Mesangial Cells were seeded at 1.3×10^5 cells per well in 6-well tissue culture dishes in DMEM + 15% Horse Serum + 1% Penicillin/Streptomycin. On following day cells were treated with different concentrations of RTA 405 for 16 hours, or RTA 405 for 16 hours followed by Ang II (1 μ M) for 30 minutes, or Ang II (1 μ M) for 30 minutes followed by RTA 405 for 16 hours. RNA was isolated using QIAshredder and RNeasy kits (Qiagen) and was reverse transcribed using iSCRIPT (Bio-Rad). qPCR was performed using SYBR Green (Bio-Rad) and Bio-Rad CFX instruments. Data was analyzed using the DDCT method and normalized to Rpl19. Figures are the average of technical replicates (n=1). Error bars are standard deviation of technical replicates. *Nqo1*: NAD(P)H dehydrogenase quinone 1; *Hmxo1*: heme oxygenase 1; *Gclc*: glutamate-cysteine ligase, catalytic subunit; *Txnrd1*: thioredoxin reductase 1.

Fig. 2S. Effect of RTA 405 treatment on the activity of Nrf2-related enzymes in rat kidney.

Male Sprague-Dawley rats were administered vehicle (sesame oil, n=3) or RTA 405 (100 mg/kg, n=5), once daily for 3 days. On Day 3, rats were euthanized 16 hours after the final dose and kidneys were removed, frozen in liquid nitrogen and stored at -80°C until analysis. NAD(P)H dehydrogenase quinone 1 (Nqo1) enzyme activity was determined by quantifying the rate of disappearance of DCPIP. Glutathione reductase (Gsr), glutathione S-transferase (Gst), and thioredoxin reductase (Txnrd) enzyme activity were determined using commercially available kits. Total GSH was determined using the Promega GSH-Glo™ assay. * denotes P<0.05 and † denotes P<0.1, compared to corresponding vehicles.

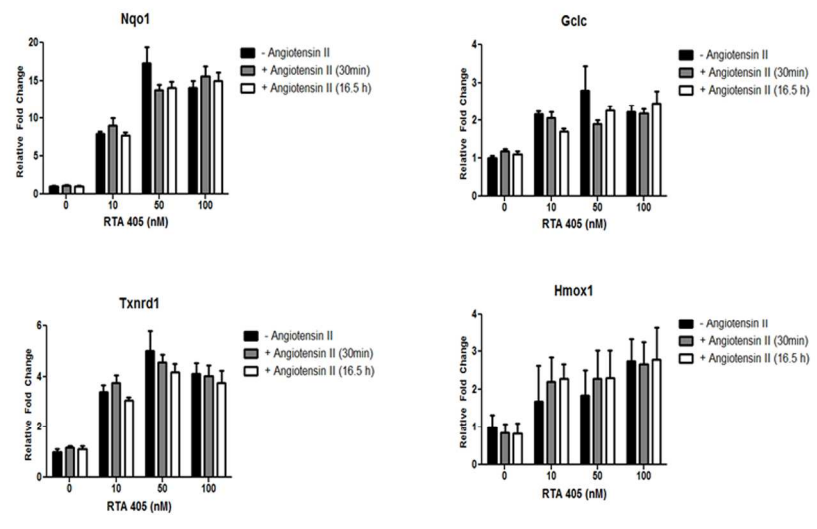


Fig. 1S

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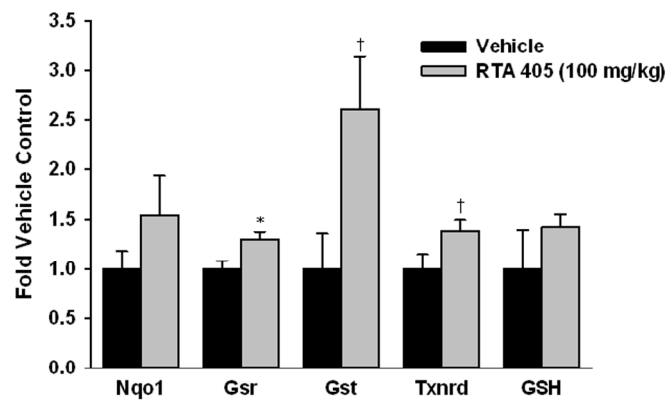


Fig. 2S

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