Supplemental Data

Methods associated with animal model preparation and other parameters measured in addition to those described below are provided in the main paper.

Urine Chemistries and Renal Biomarkers
For urine based assessments, 18 h urine samples were collected before dosing and after the highest dose to determine effects of baclofen on urine chemistries and drug-induced renal biomarkers of injury.

Urine appearance and physical characteristics (color, clarity, total volume and pH) of samples collected on Day -1 and Day 1 were recorded. Urine constituents (bilirubin, ketone, occult blood, and urobilinogen) were semi-quantitatively measured using standard dipstick technology (Siemens; New York, NY). For standard urinalysis, specimens were centrifuged at 604xg for 10 min. Baseline urine chemistry parameters (urea nitrogen, creatinine, phosphorus, and total protein), electrolytes (sodium, potassium, chloride, and calcium) were analyzed using a Hitachi® clinical chemistry analyzer (Tokyo, Japan). Renal biomarkers of injury were also evaluated using Meso Scale Diagnostic multiplex kits (Gaithersburg, MD) with results reported as a ratio to urine creatinine. Biomarkers measured included: Kidney Injury Molecule **(**KIM-1), albumin, alpha glutathione S-transferase (α-GST), mu gamma glutathione S-transferase-yb1 (GSTYβ1), lipocalin, renal papillary antigen-1 (RPA-1), clusterin and osteopontin (OPN).

Plasma Chemistries
At the end of the study, animals were humanely sacrificed and blood collected to determine effects of baclofen on plasma chemistries.

Data Anaysis
Urine electrolytes, urine chemistries, blood chemistry parameters, and biomarkers of drug induced kidney injury data were normalized to baseline (pre treatment) as a ratio then log transformed. The Student's t-test was applied to these log transformed parameters at time points indicated for comparisons (Baclofen versus vehicle). Tabular data are presented as fold changes normalized to baseline (defined as data sampled from -2 – 0 h) and subsequently compared to vehicle. Graphical data are presented as raw data without baseline correction.

Results

Plasma Chemistry and Urinalysis
Baclofen decreased levels of blood triglycerides by 40% compared to vehicle-treated rats (Supplemental Table 1) but had no effect on other blood chemistries. Baclofen also significantly ($P>0.05$) increased urine volume (220%), decreased osmolarity (-50%), decreased potassium (-14%) and total protein (-40%) compared to vehicle (Supplemental Table 2).

Drug-Induced Kidney Injury Biomarkers
Baclofen induced a significant 2-fold increase in clusterin compared to vehicle-treated animals, a change not considered physiologically significant (Supplemental Table 3). There were no other significant changes in all other measured parameters.

Supplemental Table S1. Plasma Chemistries

Plasma Chemistry Biomarker	Vehicle	Baclofen	P value
Albumin/Globulin Ratio	1.3 ± 0.2	1.5 ± 0.1	NS
Cholesterol	65 ± 5.5	65.6 ± 7.5	NS
Albumin	2.8 ± 0.1	2.9 ± 0.2	NS
Alanine AminoTransferase	26 ± 2.4	24.8 ± 2.8	NS
Alkaline Phosphatase	90 ± 38.5	83.2 ± 12.1	NS
Aspartate Transferase	82 ± 2.5	74.6 ± 3.1	NS
Total bilirubin	0.1 ± 0.04	0.06 ± 0.03	NS
Serum calcium	10.2 ± 0.4	9.5 ± 0.6	NS
Serum creatinine	0.3 ± 0.03	0.3 ± 0.03	NS
Serum glucose	164.7 ± 2.7	178.6 ± 15.2	NS
Total protein	5 ± 0.2	4.9 ± 0.4	NS
Triglycerides	90 ± 5.8	43.8 ± 9.4	*
Blood urea nitrogen	18.7 ± 3.3	17.6 ± 1.4	NS
Serum sodium	144 ± 2.5	137 ± 6	NS
Serum potassium	4.1 ± 0.4	3.8 ± 0.2	NS
Serum chloride	103.4 ± 2.3	98.5 ± 4.5	NS
Serum phosphate	6.6 ± 0.4	6.5 ± 0.4	NS
Glutamate dehydrogenase	9.7 ± 0.4	8.8 ± 0.5	NS
Globulin	2.2 ± 0.2	2 ± 0.2	NS
Serum osmolality	307 ± 0.7	312.2 ± 8.3	NS

* $p<0.05$ vs. time-matched vehicle; NS, not significant

Supplemental Table S2. Urine analysis

Urinary Chemistry Biomarker	Vehicle	Baclofen	P value
Sodium	54 ± 1	47.8 ± 4.1	NS
Potassium	37.3 ± 3.8	32.9 ± 1.7	**
Chloride	65.4 ± 6.7	47 ± 4.1	**
Glucose (mg/dL)	1.1 ± 0.7	0.8 ± 0.3	NS
Protein (g/dL)	226.7 ± 49.7	206.2 ± 43.7	**
N acetyl glucosamine	2.1 ± 0.47	2.0 ± 0.2	NS
Alanine phosphatase	0.3 ± 0.1	0.05 ± .05	NS
Lactate dehydrogenase	10.7 ± 1.5	10.5 ± 0.4	NS
Gamma-glutamyltransferase	0.3 ± 0.1	0.2 ± 0.2	NS
Volume (mL)			*
Osmolarity			*

* $p<0.05$; ** $p<0.005$ vs. time-matched vehicle; NS, not significant

Supplemental Table S3. Biomarkers of Kidney Injury

Kidney Injury Biomarkers	Vehicle	Baclofen	p value
Alpha Glutathione S-Transferase (ng/mg Cr)	1.4 ± 0.3	2.8 ± 1.0	NS
Glutathione S-Transferase. Yb1 (ng/mg Cr)	0.9 ± 0.7	0.5 ± 0.2	NS
Renal papillary antigen(U/mg Cr)	19.2 ± 6.7	15.1 ± 5.2	NS
Clusterin (U/mg Cr)	0.16 ± 0.06	0.41 ± 0.2	*
Albumin (ng/mg Cr)	511.8 ± 85	858.6 ± 401.2	NS
Lipocaln (ng/mg Cr)	37.8 ± 8.6	25.6 ± 4.9	NS
Osteopontin (ng/mg Cr)	0.08 ± 0.02	0.08 ± 0.03	NS
Kidney injury molecule-1 (ng/mg Cr)	0.14 ± 0.04	0.09 ± 0.03	NS

* $p<0.05$ vs. time-matched vehicle; NS, not significant