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Selective cyclooxygenase-2 inhibitor suppresses renal thromboxane production but not proliferative lesions in the MRL/lpr murine model of lupus nephritis

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Abstract

Background—Proliferative lupus nephritis (LN) is marked by increased renal thromboxane (TXA₂) production. Targeting the TXA₂ receptor or TXA₂ synthase effectively improves renal function in humans with LN and improves glomerular pathology in murine LN. This study was designed to address the following hypotheses: 1) TXA₂ production in the MRL/MpJ-*Tnfrsf6^{lpr}*/J (MRL/lpr) model of proliferative lupus nephritis is COX2-dependent, and 2) COX2 inhibitor therapy improves glomerular filtration rate (GFR), proteinuria, markers of innate immune response, and glomerular pathology.

Methods—20 female MRL/lpr and 20 BALB/cJ mice were divided into two equal treatment groups: 1) SC-236, a moderately selective COX2 inhibitor, or 2) vehicle. After treatment from 10 to 20 weeks of age, the effectiveness of inhibition of TXA₂ was determined by measuring urine TXB₂. Response endpoints measured at 20 weeks of age were renal function (GFR), proteinuria, urine nitrate + nitrite (NO_x), and glomerular histopathology.

Results—SC236 therapy reduced surrogate markers of renal TXA₂ production during early, active glomerulonephritis. When this pharmacodynamic endpoint was reached, therapy improved GFR. Parallel reductions in markers of the innate immune response (urine NO_x) during therapy were observed. However, the beneficial effect of SC236 therapy on GFR was only transient, and renal histopathology was not improved in late disease.

Conclusions—These data demonstrate that renal TXA₂ production is COX2-dependent in murine LN and suggest that NO production is directly or indirectly COX2-dependent. However, COX2 inhibitor therapy in this model failed to improve renal pathology, making COX2 inhibition a less attractive approach for treating LN.

Keywords

Lupus nephritis; animal models; cyclooxygenase 2; nitric oxide; glomerular filtration rate; thromboxane A₂

Introduction

Lupus nephritis is an immune complex-mediated glomerulonephritis that affects approximately 50% of patients with systemic lupus erythematosus (SLE). Proliferative nephritis, the most common type of lupus nephritis, is marked by immune complex deposition in the subendothelial space and a robust innate and cellular immune response. On histopathology, this lesion is characterized by cellular infiltration, proliferation of intrinsic cell types, and destruction of glomerular capillary loops with a more rapid decline in renal function than other types of lupus nephritis (1). Part of this robust innate immune response in humans and murine models of proliferative LN is increased production of thromboxane A₂ (TXA₂), the product of TXA₂ synthase (TXA₂S) (2,3). TXA₂ is a pro-inflammatory, vasoconstricting lipid mediator. In platelets, the prostaglandin H₂ (PGH₂) substrate of TXA₂S is cyclooxygenase 1 (COX1)-dependent. In inflamed tissue, PGH₂ production is often dependent on the inducible form of cyclooxygenase, COX2, and the cell source is often macrophages or fibroblasts (4).

There is a longstanding interest in targeting TXA₂ in LN, as several studies have demonstrated a potential pathogenic role for this mediator. TXA₂ receptor antagonist (TXA₂RA) antagonist and TXA₂S inhibitor therapy have improved endpoints such as renal function, glomerular pathology, and survival in a murine model of lupus nephritis (5–8). In humans, short-term treatment with a TXA₂RA induced transient improvements in renal function, suggesting that these findings could be translated to human disease (9). However, TXA₂RA therapy was complicated by a doubling in bleeding time, suggesting that both platelet and inflammatory cell TXA₂ production was inhibited. This study demonstrated no improvement in renal function with low dose aspirin, suggesting that therapy selective for inflammatory cells but sparing platelets would be equally efficacious. One way to accomplish this selectivity for inflammatory cells would be to target COX2-dependent PGH₂ production. Previous studies testing COX2 inhibitors in murine lupus nephritis showed efficacy only in the NZB/W model when used in combination with immunosuppressive therapy. These findings had not been confirmed in an alternate model of lupus nephritis.

This study was designed to address the following hypotheses: 1) TXA₂ production in the MRL/MpJ-*Tnfrsf6*^{lpr}/J (MRL/lpr) model of proliferative lupus nephritis is COX2-dependent, and 2) COX2 inhibitor therapy will improve glomerular filtration rate (GFR), proteinuria, markers of innate immune response, and glomerular pathology in this murine model of lupus nephritis.

Methods

Mice and sample collection

The Ralph H. Johnson VA Institutional Animal Care and Use Committee approved all procedures performed in this protocol. MRL/MpJ-*FAS*^{lpr}/J (MRL/lpr) and BALB/cJ mice (as a non-disease control for renal pathology) were purchased from Jackson Laboratory (Bar Harbor, ME) and housed under specific pathogen-free conditions in the animal research facility at the Ralph H. Johnson Veterans Affairs Medical Center in Charleston, SC. Mice were serologically tested for common murine pathogens on a random basis. Ten mice from

each strain were treated with SC-235, while the remaining ten mice received vehicle. Every two weeks, before (at 10 weeks of age) and during treatment, five individual mice from each group were placed on a 24-hour low nitrate + nitrite (NO_x) diet (Zeigler Brothers, Gardners PA) with distilled water to prevent dietary contamination of urine NO_x measures. Mice were then placed in metabolic cages for an additional 24 hours on the same diet/water for urine collection (10). Throughout the remainder of the experiment, mice were fed standard mouse chow and tap water. Five separate mice from each group were evaluated for glomerular filtration rate (GFR) by clearance of ^{51}Cr labeled ethylenediaminetetraacetic acid (EDTA) (11). At 19–20 weeks of age, mice were anesthetized for bleeding from the retro-orbital plexus and killed for harvest of renal tissue (10).

Treatment

Twenty MRL/lpr and 20 BALB/cJ mice were each divided into two, equal treatment groups: 1) SC-236 ([4-[5-(4-chlorophenyl)-3-(trifluoromethyl)-1-pyrazol-1-yl], Skokie, IL) 2 mg/kg three times a week in vehicle with sweetener (0.5% polyethylene glycol, 5% Tween 20, and saccharin (2.67 g/L, Sweet'N Low, Cumberland Packing Corp., Brooklyn, NY) in distilled water) ad libitum in drinking water with or 2) sweetened vehicle alone in drinking water. Mice were treated from 10 weeks of age to 20 weeks of age. Mice were monitored for PO liquid intake to ensure proper dosing. This dose was chosen based on studies indicating effective selective COX2 inhibition at this dose in rodents. The half-life of SC236 is 177 hours in rats (12). The IC_{50} of SC236 is 5 nM for COX2 and 17 μM for COX1 (13).

Urine protein

Urine collected and stored as above was analyzed at 1:10 dilution in batch analysis for total protein content in duplicate using the BioRad modified Lowry assay per the manufacturer's protocol. Results were reported as mg/mouse/day.

Measurement of urine thromboxane B_2 (TXB_2)

Thromboxane A_2 of renal origin is rapidly metabolized to TXB_2 and excreted in the urine. Urine from 24-hour urine collections ($n = 5$) every other week of treatment were centrifuged and frozen at -80°C . Samples were batch analyzed using the Cayman TXB_2 RIA kit (, Ann Arbor, MI). Results were expressed as ng/mouse/day.

Urine Nitrate + Nitrite (NO_x) assay—Twenty-four hour urine samples ($n=5$) were collected into 1 ml of an antibiotic solution containing 25 $\mu\text{g}/\text{ml}$ ampicillin, 50 $\mu\text{g}/\text{ml}$ gentamicin, and 200 $\mu\text{g}/\text{ml}$ chloramphenicol to prevent bacterial growth. The samples were centrifuged at 2500g to remove debris and frozen at -80°C . Samples were thawed in batch and filtered through a 0.22 μm centrifugal filter (Millipore, Bedford, MA). NO_x was assayed using a chemiluminescence detector (Model 280i, Sievers, Boulder, CO) with vanadium chloride at 95°C as a reducing agent as described (9). Unknown sample concentrations were interpolated from curves generated from nitrate standards (Sigma-Aldrich, St. Louis MO) by the manufacturer's specifications. All samples were assayed in duplicate or triplicate (if > 10% coefficient of variation was noted in duplicates) and reported as $\mu\text{moles}/\text{mouse}/\text{day}$.

EDTA clearance assay

GFR was measured by disappearance of EDTA from plasma EDTA ($n=5$), a measure that correlates closely with inulin clearance as a surrogate for GFR (1,2,3). ^{51}Cr EDTA was chelated to $^{51}\text{CrCl}$ in 0.1 M Na EDTA, pH 3.5. ^{51}Cr EDTA, ($13-15 \times 10^6$ cpm). This chelated agent was given subcutaneously at time zero. Timed tail vein nick blood samples were collected at 15, 30, 60, 90, 120, 180, 240 minutes after injection into a volume-calibrated capillary tube (Catalog # 02-068-66 75mm, Fisher, Pittsburgh, PA). The blood

volume and plasma volume (using estimated hematocrit) were calculated, and the capillary tube [^{51}Cr] was analyzed for gamma counts. An aliquot of the ^{51}Cr EDTA solution given to the mice was counted at the same time as the blood samples to determine the CPM per given dose. The clearance was calculated as the dose administered/area under the plasma disappearance curve ($\text{Clearance} = \text{Dose}/\text{AUC}$) (11).

Qualitative glomerular scores

At the end of treatment, mice were killed for harvest of cortical tissue ($n=13$), which was immediately fixed in paraformaldehyde and stained with hematoxylin and eosin before examination by a pathologist (PR) who was masked to the treatment group. Pathologic changes of renal disease activity were graded qualitatively as previously published (14). Factors scored in the determination were hypercellularity, mesangial expansion, hyperlobulation, chronic inflammation, dilatation, epithelial cell reactivity, thrombi in loops, vasculitis, thickened membranes, and neutrophil debris, necrosis, and crescent formation. Scores can range from zero to a maximum of 60.

Statistical analysis

Single time-point comparisons between groups were made using Student's t-test or Mann-Whitney rank sum analysis depending on the distribution of the data. ANOVA was used to test for differences between groups over time. Differences were considered significant if p values were ≤ 0.05 . All data were reported as a mean $\pm$ standard error.

Results

Urine TXB_2 and nitrate+nitrite (NO_x) levels increased as renal disease progressed in MRL/lpr mice

Untreated MRL/lpr mice were observed from prior to disease onset (at 10 weeks) to full disease expression (20 weeks). Urine was collected in metabolic cages every two weeks and analyzed for nitrate+nitrite (NO_x a measure of the innate immune response) and TXB_{2a} . Urine TXB_2 levels (reflecting renal TXA_2 production) significantly increased in parallel with the progression of renal disease (as indicated by rising urine NO_x and urine protein levels) in MRL/lpr mice (Figure 1A and B). Neither TXB_2 nor NO_x levels rose with age in BALB/cJ mice (data not shown). This suggests that either the activity of TXA_2 synthase was increased with disease activity or that disease activity increased the production of the common substrate, PGH_2 . It was unclear from these observations alone from which isoenzyme of cyclooxygenase (COX1 or COX2) PGH_2 substrate was produced as disease progressed. The parallel increase in urine NO_x suggests either common upstream mechanisms or crosstalk between COX-dependent pathways and NO production.

Increased renal TXA_2 production was COX2-dependent in MRL/lpr mice

SC236 is a selective COX2 inhibitor. MRL/lpr mice were treated with either SC236 or vehicle from 10 to 20 weeks of age. Treatment with SC236 was associated with significantly lower urine TXB_2 levels in MRL/lpr mice (Figure 2A) suggesting that excessive renal TXA_2 production in this model of lupus was COX2-dependent. Urine TXB_2 levels were not affected by therapy in BALB/cJ mice (data not shown). Serum TXB_2 levels (indicative of non-renal TXA_2 production) were significantly higher in control compared to SC236 treated MRL/lpr mice (304 ± 36 vs. 156 ± 23 , $p = 0.003$). Taken together, these data suggest that the rise in renal TXA_2 production seen with disease activity in MRL/lpr mice is COX2-dependent. It also suggests that there is a component of COX2-dependent non-renal TXA_2 production in MRL/lpr mice.

COX2 inhibitor therapy reduced systemic NO production in MRL/lpr mice

In control mice, urine NO_x, as a surrogate measure of systemic NO production, increased as disease progressed. This effect has been described previously and was expected (10,15,16). Mice treated with SC236 had significantly lower urine NO_x levels than control mice (Figure 2B). Urine NO_x levels were not affected by therapy in BALB/cJ mice. This observation suggests that NO production was either directly or indirectly dependent on COX2 activity with disease progression in MRL/lpr mice.

COX2 inhibitor therapy transiently improved Cr⁵¹EDTA clearance but did not improve urine protein levels or glomerular pathology in MRL/lpr mice

Cr⁵¹EDTA clearance was measured as a surrogate for glomerular filtration rate (GFR) in MRL/lpr mice before and five and 10 weeks after initiation of treatment with either SC236 or vehicle. Cr⁵¹EDTA clearance was significantly greater in SC236-treated MRL/lpr mice five weeks after initiation of therapy in association with an increase in proteinuria at this time point (Figure 2C). This improvement was transient, as Cr⁵¹EDTA clearance was similar in both groups after 10 weeks of therapy (Figure 2D). GFR was unaffected by age or treatment in BALB/cJ mice. While SC236 mice had lower urine NO_x levels, suggesting a reduced innate signaling in the kidney, renal pathology from 20-week-old treatment and control mice was similar (total glomerular scores 8.9 ± 1.7 vs. 9.8 ± 1.6 , respectively). This would suggest that improvements in GFR with SC236 therapy were not due to reduced cellular infiltrates and hypercellularity but rather due to improvements in glomerular perfusion that associate with reduced renal TXA₂ production. Similarly, transient rises and proteinuria can be observed with rises in GFR independent of glomerular pathology.

Discussion

In this model of lupus nephritis, SC236 therapy reduced surrogate markers of renal TXA₂ production during early, active glomerulonephritis. When this pharmacodynamic endpoint was reached, therapy successfully improved a surrogate marker of GFR. Parallel reductions in markers of the innate immune response (urine NO_x) during therapy were observed. However, the beneficial effect of SC236 therapy on GFR was only transient, and renal histopathology was not improved in late disease.

Two previous studies have evaluated the effect of COX2 inhibitors on an alternative model of lupus nephritis, the (NZBxNZW)_{F1} (NZB/W) model (17,18). In one study, low dose, intermittent celecoxib therapy resulted in reduced glomerular hypercellularity and crescent formation on histopathology at 18 weeks, which represents early disease in this model. This is consistent with our observation that GFR was improved early in disease. However, because we did not evaluate histopathology at this early stage, we cannot conclude that therapy transiently improved glomerular pathology at that time point in our study. In addition to improving pathology, celecoxib therapy resulted in modest reductions in levels of disease-relevant autoantibodies, an effect seen only when low, intermittent dosing was administered (18). This would suggest that COX2 inhibitor therapy affects lymphocyte function in addition to innate and cellular immune responses in the glomerulus. In another, much larger study, NZB/W mice were administered a COX2 inhibitor (5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5H)-furanone or DFU) alone or in combination with mycophenolate mofetil (MMF). DFU therapy only provided an advantage in survival or reduced proteinuria when in combination with MMF. However, DFU monotherapy did result in reduced intra- and extra-capillary hypercellularity and interstitial damage at nine months, which represents more advanced disease in this model. In our study, hypercellularity was reduced slightly (2.3 ± 0.6 vs. 2.8 ± 0.8) but not significantly ($p = 0.12$), indicating that COX2 inhibition is not as effective at preventing proliferative lesions in the

more aggressive MRL/lpr model. In a study of NZB/W mice, TXA₂ production was increased in isolated spleen cells, an effect that was reduced with DFU therapy (17). This is consistent with our observation that COX2 inhibitor therapy led to reduced serum TXB₂ levels. This suggests extra renal TXA₂ production is COX2 dependent in the MRL/lpr model as well.

TXA₂ remains a viable target for therapy in lupus nephritis. However, the most effective fashion for targeting its production in proliferative nephritis (COX2 inhibition vs. TXA₂S inhibition or antagonism of the TXA₂R) with the least side effects is not known. Translation of these findings to the use COX2 inhibitor therapy in humans with lupus nephritis is complicated by potential side effects and the inconsistent improvements in renal pathology in the aforementioned murine studies. The observation that GFR was improved early in therapy is consistent with human studies showing an improvement in GFR with acute therapy (9). Use of COX2 inhibitors in acute glomerulonephritis is complicated by two combined observations. First, elevated levels of anti-phospholipid antibodies are more prevalent in lupus patients. Second, thrombotic events have been observed in connective tissue disease patients with antiphospholipid syndrome immediately after initiation of COX2 inhibitor therapy (19). MRL/lpr (20) NZB/W (21,22) mice both produce anti-cardiolipin antibodies, but thrombosis was not observed with COX2 inhibitor therapy in any of these studies. However, this negative finding should be interpreted with caution given that thrombosis was not studied as a formal endpoint.

Chronic therapy could be complicated by the potential for increased cardiovascular complications in the lupus population. First, hypertension has been observed in non-nephritis patients given COX2 inhibitor therapy (23). Blood pressure was not monitored in any of these three murine studies, making it difficult to extrapolate to humans with lupus nephritis. Our observation that GFR was transiently improved early in disease and was not reduced late in disease with SC236 therapy would argue that, if it did occur, the effects on glomerular function were not clinically significant. However, this hypothesis would need to be tested with direct measurements in the setting of lupus nephritis for a definitive conclusion. Another factor that reduces enthusiasm for chronic COX2 inhibitor therapy is the known effects of chronic COX2 inhibitor therapy on cardiovascular events in populations at high risk for such events (24). Mechanisms for the delayed onset of increased mortality with high dose COX inhibitors is not known, but cholesterol efflux from foamy macrophages is known to be COX2 dependent (25). Lupus is an independent risk factor for cardiovascular disease, but models for predicting those lupus patients at risk for stroke and myocardial infarction are imperfect (26). One way to avoid this complication might be to use of nonselective COX inhibitors that inhibit COX1-dependent platelet activity. However, earlier studies demonstrated that indomethacin reduced proteinuria in lupus patients with refractory proteinuria but caused side effects such as hypertension and reduced glomerular function (27,28). This effect may have stemmed from inhibition of COX1-dependent prostacyclin (analogs of which are known to increase GFR and reduce proteinuria in LN (29)) in addition to COX2-dependent TXA₂ (known to reduce GFR (5)). Thus, these side effects would prohibit therapeutic use in humans.

The combined data from our study and those of others (17,18) demonstrate that COX2 inhibitor therapy has a modest effect on early proliferative glomerulonephritis in two murine models of lupus nephritis but does not consistently result in improvement in hard endpoints such as survival and renal histopathology late in disease unless combined with MMF. Translation of these findings to patients with lupus nephritis is complicated by the increased thrombotic and cardiovascular risk factors seen in lupus patients.

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References

1. D'Agati, VD.; Appel, GB. Lupus Nephritis: pathology and pathogenesis. In: Wallace, DJ.; Hahn, B.; Dubois, EL., editors. Dubois' lupus erythematosus. Philadelphia: Lippincott Williams & Wilkin; 2007. p. 1094-111.
2. Kelley VE, Sneve S, Musinski S. Increased renal thromboxane production in murine lupus nephritis. *J Clin Invest* 1986;77(1):252-9. [PubMed: 3455932]
3. Patrono C, Pierucci A, Salvati P. The role of thromboxane A2 in experimental and human models of chronic glomerular damage. *Adv Prostaglandin Thromboxane Leukot Res* 1989;19:199-202. [PubMed: 2526467]
4. Seibert K, Zhang Y, Leahy K, et al. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proc Natl Acad Sci U S A* 1994 Dec 6;91(25):12013-7. [PubMed: 7991575]
5. Spurney RF, Bernstein RJ, Ruiz P, et al. Physiologic role for enhanced renal thromboxane production in murine lupus nephritis. *Prostaglandins* 1991;42(1):15-28. [PubMed: 1771236]
6. Spurney RF, Fan PY, Ruiz P, et al. Thromboxane receptor blockade reduces renal injury in murine lupus nephritis. *Kidney Int* 1992;41(4):973-82. [PubMed: 1387435]
7. Kawakage M, Mizumoto H, Nukui E, et al. Effects of KW-3635, a specific thromboxane A2-receptor antagonist, on the development of lupus nephritis in NZB x NZW F1 mice. *Jpn J Pharmacol* 1993;63(4):433-8. [PubMed: 8121078]
8. Salvati P, Lamberti E, Ferrario R, et al. Long-term thromboxane-synthase inhibition prolongs survival in murine lupus nephritis. *Kidney Int* 1995;47(4):1168-75. [PubMed: 7783415]
9. Pierucci A, Simonetti BM, Pecci G, et al. Improvement of renal function with selective thromboxane antagonism in lupus nephritis. *N Engl J Med* 1989;320(7):421-5. [PubMed: 2643773]
10. Njoku C, Self SE, Ruiz P, et al. Inducible nitric oxide synthase inhibitor SD-3651 reduces proteinuria in MRL/lpr mice deficient in the NOS2 gene. *J Invest Med* 2008 Oct;56(7):911-9.
11. Hackbarth H, Hackbarth D. Genetic analysis of renal function in mice. 1. Glomerular filtration rate and its correlation with body and kidney weight. *Lab Anim* 1981 Jul;15(3):267-72. [PubMed: 7289578]
12. Loftin CD, Trivedi DB, Langenbach R. Cyclooxygenase-1-selective inhibition prolongs gestation in mice without adverse effects on the ductus arteriosus. *J Clin Invest* 2002 Aug;110(4):549-57. [PubMed: 12189249]
13. Gierse JK, McDonald JJ, Hauser SD, et al. A single amino acid difference between cyclooxygenase-1 (COX-1) and -2 (COX-2) reverses the selectivity of COX-2 specific inhibitors. *J Biol Chem* 1996 Jun 28;271(26):15810-4. [PubMed: 8663121]
14. Sekine H, Graham KL, Zhao S, et al. Role of MHC-linked genes in autoantigen selection and renal disease in a murine model of systemic lupus erythematosus. *J Immunol* 2006 Nov 15;177(10):7423-34. [PubMed: 17082662]
15. Njoku CJ, Patrick KS, Ruiz P Jr, et al. Inducible nitric oxide synthase inhibitors reduce urinary markers of systemic oxidant stress in murine proliferative lupus nephritis. *J Invest Med* 2005 Nov;53(7):347-52.
16. Oates JC, Ruiz P, Alexander A, et al. Effect of late modulation of nitric oxide production on murine lupus. *Clin Immunol Immunopathol* 1997 Apr;83(1):86-92. [PubMed: 9073540]
17. Zoja C, Benigni A, Noris M, et al. Mycophenolate mofetil combined with a cyclooxygenase-2 inhibitor ameliorates murine lupus nephritis. *Kidney Int* 2001;60(2):653-63. [PubMed: 11473648]
18. Zhang L, Bertucci AM, Smith KA, et al. Hyperexpression of cyclooxygenase 2 in the lupus immune system and effect of cyclooxygenase 2 inhibitor diet therapy in a murine model of systemic lupus erythematosus. *Arthritis Rheum* 2007 Dec;56(12):4132-41. [PubMed: 18050205]

19. Crofford LJ, Oates JC, McCune WJ, et al. Thrombosis in patients with connective tissue diseases treated with specific cyclooxygenase 2 inhibitors - A report of four cases. *Arthritis Rheum* 2000;43(8):1891–6. [PubMed: 10943882]
20. Gharavi AE, Pierangeli SS, Harris EN. Origin of antiphospholipid antibodies. *Rheum Dis Clin North Am* 2001 Aug;27(3):551–63. [PubMed: 11534259]
21. Kita Y, Sumida T, Ichikawa K, et al. V gene analysis of anticardiolipin antibodies from MRL-lpr/lpr mice. *J Immunol* 1993 Jul 15;151(2):849–56. [PubMed: 8335914]
22. Kier AB. Clinical neurology and brain histopathology in NZB/NZW F1 lupus mice. *J Comp Pathol* 1990 Feb;102(2):165–77. [PubMed: 2324339]
23. Muscara MN, Vergnolle N, Lovren F, et al. Selective cyclo-oxygenase-2 inhibition with celecoxib elevates blood pressure and promotes leukocyte adherence. *Br J Pharmacol* 2000;129(7):1423–30. [PubMed: 10742298]
24. Juni P, Nartey L, Reichenbach S, et al. Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. *Lancet* 2004 Dec 4–10;364(9450):2021–9. [PubMed: 15582059]
25. Reiss AB, Anwar F, Chan ES, et al. Disruption of cholesterol efflux by coxib medications and inflammatory processes: link to increased cardiovascular risk. *J Investig Med* 2009 Aug;57(6):695–702.
26. Hahn BH. Systemic lupus erythematosus and accelerated atherosclerosis. *N Engl J Med* 2003 Dec 18;349(25):2379–80. [PubMed: 14681501]
27. Espinoza LR, Jara LJ, Martinez-Osuna P, et al. Refractory nephrotic syndrome in lupus nephritis: favorable response to indomethacin therapy. *Lupus* 1993 Feb;2(1):9–14. [PubMed: 8485564]
28. ter Borg EJ, de Jong PE, Meijer S, et al. Renal effects of indomethacin in patients with systemic lupus erythematosus. *Nephron* 1989;53(3):238–43. [PubMed: 2677808]
29. Clark WF, Parbtani A, McDonald JW, et al. The effects of a thromboxane synthase inhibitor, a prostacyclin analog and PGE1 on the nephritis of the NZB/W F1 mouse. *Clin Nephrol* 1987 Dec; 28(6):288–94. [PubMed: 2450713]

Figure 1. Urine TXB₂, NO_x, and protein levels increase with age in MRL/lpr mice

24-hour urine samples from five MRL/lpr mice were collected in metabolic cages every other week from the age of 10 to 20 weeks. Urine was analyzed for TXB₂ (reflective of renal TXA₂ production), nitrate+nitrite (NO_x, reflective of systemic NO production), and protein. * $p < 0.05$ for changes in variable by week using ANOVA.

Figure 2. Urine TXB₂ and NO_x are suppressed with SC236 therapy in association with transient increases in GFR

Ten MRL/lpr mice were divided into equal groups and treated with either SC236 (2 mg/kg every other day) or vehicle from 10 to 20 weeks of age. Urine from 24-hour collections was analyzed for A. TXB₂, B. NO_x, and C. protein every two weeks after a baseline collection at 10 weeks of age. D. GFR was measured by blood clearance of ⁵¹CrEDTA at 15, and 20 weeks of age after a baseline was established at 10 weeks of age. * $p < 0.05$ for changes in variable by treatment using Mann-Whitney U test. ** $p < 0.05$ for 5 week observation only.